Method Development for Analysis of Drugs and Forensic Interest

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This study reports with the sole purpose to analyze the forensic interest compounds and drugs. The scope of analysis covers some plant poisons; some agricultural products which include pesticides, herbicides, and fungicides.Most of these compounds received a fair amount of attention as to method development and adaptation of equipment for the optimal detection of forensic interest compounds.The study has elevated the analysis of drugs and forensic interest compounds for most of the received cases in FSL. The developed methods were quite unique as no published methods could be found that was applied for the analysis of pesticides. The method developed covers the classes of plant poisons as well as the most commonly available and widely used pesticides.The approach and methods of extraction, detection and analysis developed during this Study can be further expanded to cover a wider range of pesticides for each of the main Classes of compounds (Organochloro, phosphorous, carbamates, pyrithroids, organotin). An attempt is made to plant-related as well as newly invented pesticide forensic analysis in FSL can become more sophisticated, with a higher success rate in terms of the number.

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“METHOD DEVELOPMENT FOR ANALYSIS OF DRUGS AND FORENSIC INTEREST COMPOUNDS FROM BIOLOGICAL AND NON-BIOLOGICAL MATERIALS”

ABSTRACT

Forensic science is the application of a broad spectrum of sciences to answer questions of interest to a legal system. Thismay be in relation to a crime or a civil action. Forensic toxicology has developed as a forensic science in recent years and is now widely used to assist in death investigations, in civil and criminal matters involving drug use, pesticides and other toxic compounds, plants toxins etc. The incidence of poisoning in India is among the highest in the world, and it is estimated that more than 50,000 people die every year from toxic exposure. The causes of poisoning are many - civilian and industrial, accidental and deliberate. The commonest agents of poisoning in India appear to be pesticides (organophosphates, carbamates, chlorinated hydrocarbons, and pyrethroids), sedative drugs, drugs most commonly targeted include amphetamines, benzodiazepines, cannabis, cocaine and the opiates, but can be any other illicit substance or almost any over-the-counter or prescribed drug, as well as poisons available to the community. chemicals (corrosive acids and copper sulphate), alcohols, plant toxins (datura, oleander, strychnos, and gastro-intestinal irritants such as castor, croton, Calotropis, etc.), and household poisons (mostly cleaning agents). Therefore, with the ever-increasing cases of poison in India, the role of forensic toxicology has been greatly appreciated in various cases. The discipline requires high level skills in analytical techniques with a solid knowledge of pharmacology and pharmacokinetics and chemistry.

Poisoning has become one of the commonest medical emergencies throughout the world because thousands of pharmacological and chemical agents are commonly used and their numbers are increasing every year. There is tremendous rise in the use of insecticide, pesticide and other potentially poisonous substances in last five years for the purpose of crop protection, but their misuse and easy availability has led to suicidal, homicidal or accidental poisoning cases. Every year, more than 10,000 fatal poisoning cases received in forensic science laboratory as, toxicological analysis is constantly increasing.The Forensic toxicology testing allows forensic scientists to identify substances and determine a pattern of use.

The author, as a part of forensic science laboratory, tried to analyse such type of cases. Accurateanalysis ofpoisons in poisoning case is essential, both in the living as well as in the dead, for therapeutic and medicolegal purposes respectively. Poisons are generally detected in biological materials- (body fluids such as urine, blood, or gastric lavage) during life, while they are detected in the contents of stomach, bowel and the viscera, besides urine and vomitus, after death. Poisons are also detected in non-biological materials such as containers, bottles, soil, food, unknown powders, crime scene evidences etc.

The poison isolated from biological material in poisoning cases generally is in microgram quantities. The conventional methods of chemical analysis are not feasible in such cases; hence micro chemical and instrumental technique has to be developed for detection, isolation and quantitation of isolated poison.

Literature survey reveals that Preliminary examination of materials may include chemical spot tests which by colour production may indicate the existence of a type of drug or poison. More sophisticated techniques include Ultraviolet Absorption (UV), Infrared Absorption (IR), Radioimmunoassay (RIA), Thin-Layer Chromatography (TLC), High Performance Liquid Chromatography (HPLC), and Gas Chromatography (GC) are available. The application of Mass Spectroscopy (MS) is now commonly employed for identification of drug and toxic compounds.

A number of analytical and advanced instrumental methods, i.e., gas chromatography, gas chromatography and mass spectroscopy, as well as high-performance liquid chromatography have been reported for the easy detection of pesticides from biological and non-biological materials. Though these techniques are rapid, specific, and sensitive, they cannot be always used for the detection of insecticides which are extracted from biological materials (in autopsy tissues), as the purity of samples is in question.

Another major thing is that, when patient is admitted in hospital for treatment of poisoning, his/her urine, blood, or gastric lavage received by FSL for accurate detection and treatment for poison becauseMedical officer can’t give correct treatment without knowing the nature of poison. Majority of patient die due to incorrect

treatment. For correct treatment, Identification of these pesticide and insecticide-poisons is of great importance. In Rural area of Maharashtra there is unavailability of institute or laboratories which carry the Identification of poison, to facilitate the proper treatment of the victim. Forensic toxicologists have great challenge to analyse such type of cases for these purpose simple, rapid, cheap and innovative techniques should be developed.In the present study, TLC and HPTLC techniques was found to be sensitive techniques for detection and Identification of poison from biological materials.

The poison isolated from biological material in poisoning cases generally is in microgram quantities. For identification of these substances an attempt has been made to develop new techniques by using Thin Layer Chromatography (TLC) and HPTLC (High-performance thin-layer chromatography). These methods are highly sensitive and can be used for identification of pesticides, /drugs, etc. these methods are discussing in in present study. An attempt is being made to develop a new chromogenic reagents and analytical methods for selective detection of pesticides and drugs of forensic interest.

The procedures used in a typical toxicology section of a laboratory may include extraction and purification techniques prior to analysis. In our present study, Classical method for extraction of non-volatile organic poison, Modern method for extraction of non-volatile organic poison, Solvent extraction, Stas-Otto method, Solid-phase extraction (SPE), Accelerated Solvent Extractor has been described in detail.

Following the general introduction and literature survey in Chapter I, Development of method for isolation and extraction of drugs and pesticides from biological materials (Chapter II) is described, as well as analysis of pesticides (Chapter II, Section A, and B) focuses on new chromogenic reagents. wedeveloped, A New Chromogenic Reagent for Carbamate Insecticides. In the present study, the authors have made efforts to use a new chromogenic spray reagent for the high-performance thin-layer chromatographic detection of carbamates. In this work, we used 10% aq. sodium hydroxide followed by 5% of toluidine reagent and 10% of aq.

sodium nitrate, giving orange and violet spots. The biological impurities such as amino acids, peptides etc. present in visceral material do not interfere in the test.

In Chapter II, section B, we developed A Specific Spray reagent for the identification and detection of carbaryl in biologicalmaterials. In present work, we reported, the use of 10% NaOH solution followed by a mixture of sodium bromide and copper chloride for high-performance thin-layer chromatographic (HPTLC) detection and identification of carbaryl insecticide with a solvent system hexane and ethyl acetate (9:1).

In Chapter III, section A, the study combined, for the first time, forensic investigation, chemistry and botany to create a unique platform needed for the identification of poisonous plants and their components in forensic exhibits, blood, urine, stomach wash and viscera. The research was focused on the poisonous plants previously detected at the laboratory, as well as the requests received for the analysis of multi/toxic plant components. The selection of plants included Nicotiana glauca, Datura stramonium / Datura ferox, Callilepis laureola, Boophone disticha / Ammo Charis coranica, Abrus precatorius, Ricinus communis, Nerium oleander / Thevetin peruviana and Bowiea volubilis. All these species are known to have caused fatalities, hence their choice.

A lot of work has been reported on identification and detection of poisonous plant but no work has been done in terms of forensic context. In this research, we reported a data in which, basic detail such as botanical and family name, toxic part of plant, chemical constituents, fatal dose and fatal period which is given in paper. Effort has been taken to overcome the method of detection of poison plants, spray reagents, mobile phase and sensitivity.

In Chapter III, Section B, we developed method for detection of Cannabis by HPTLC. Although the instrumental methods are sensitive, they are expensive but there are limitations to their use in routine forensic work owing to the large number of samples (involving urine samples) to be handled. In this study we found that HPTLC method was found to be high -throughput, sensitive, reproducible and cost-effective compared to other methods. A number of chromogenic reagents have been reported.

In a search for an alternative chromogenic reagent, P-Anisidine reagent in combination with ammonium metavanadate was found to be suitable for the detection of cannabinoids in marijuana.

Development of method for isolation and identification of newly invented pesticideshave been described in chapter IV.In section A, Acephate is an insecticide that belongs to the organophosphate group of chemicals. We developed a new, specific chromogenic spray reagent and new solvent system for the detection and identification of Acephate by HPTLC.The reagent consisting of 0.1% solution of ferric chloridein 80% ethanol with 1% Sulfosalicylic acid in 80% ethanol.Solvent system-(petroleum ether: methanol 95:5).

Describing section B, the increasing number of biological samples for poison detection, there is a need of versatile, sensitive and selective reagent. In a search for a selective and sensitive reagent, alkaline hydrolysis of pyridine with p-amino azobenzene was found to be suitable for detection and identification of Endosulfan in routine forensic toxicological analysis. High Performance Thin Layer Chromatography (HPTLC) is the method of choice because of its speed, low cost and versatility.

In the present paper, we report the use of 5% NaOH solution and 2% pyridine followed by *p*-amino azobenzene in acetic acid yielding intense orange colour. For better analysis, various solvent systems were used.HPTLC Detection of Pyrethroids in Autopsy Tissues, an attempt has been made in section C. We developed,alkaline hydrolysis of p-nitro benzaldehyde as a specific spray reagent for α cyno ester by High Performance Thin Layer Chromatography. The basic of this reagent underlines on the formation of well-known chemical reaction of Benzoin condensation. This reagent produces violet spots relatively with synthetic Pyrethroids containing cyno group.

In Chapter V, application of research work in solving forensic case work has been described. Two case studies have been solved by using new spray reagents.Hence in the presentstudy , an author, being a part of forensic laboratory found that there are so many problems for identification of various poisoning

substances in biological and non-biological materials. There are no previous references for identification of such types of cases. By doing research and development work, and using the spray reagent developed by us, these cases are solved. Thus, the innovative methods applied by the author facilitated the identification of modern-day insecticides and pesticides. This is the boost to forensictoxicology division to enhance the analytical methodologies by which the challenge of finding out modern day poisons can be achieved.

Ulka Krishna Kulkarni (Research Student)

Dr. Dhananjay V. Mane (Research Guide)

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Mrs.Ulka Krishna Kulkarni

Contents

ABBREVIATIONS

b.p	-	Boling point
BHC	-	Hexachlorocyclohexane
cm	-	Centimetre
ca	-	about
DDT	-	Dichloro dimethyl trichloethane
DNA	-	Deoxyribonucleic acid
HCB	-	Hexachlorobenzene
HPLC	-	High Performance Liquid Chromatography
GC-MS	-	Gas Chromatography-Mass Spectrometry
GC-FID	-	Gas Chromatography- Flame Ionization Detector
GC-ECD	-	Gas Chromatography- Electron Capture Detector
FAO	-	Food agriculture organization
FC	-	Folin Ciacaltau
FTIR	-	Fourier Transform Infrared Spectroscopy
LC-MS	-	Liquid Chromatography-Mass Spectrometry
LD_{50}	-	Lethal Dose
LOD	-	Limit of detection
LOQ	-	Limit of detection
m.p	-	Melting Point
mg	-	Milligram
mm	-	Millimetre
mg / kg	-	Milligram per kilogram
OP	-	Organophosphorus
OC	-	Organochlorine
ppm	-	Parts per million
ppb	-	Parts per billion
TLC	-	Thin layer chromatography
UV	-	Ultra violet
WHO	-	world health organization

Chapter 1

Aim of Study and Introduction

Section A

Section B

1.Aim of Study:

This study is the first phase of an ongoing research project initiated to promote and enhance Forensic Chemistry in India. It is a multidisciplinary investigation combining forensic investigation, chemistry and forensic toxicology to create a unique platform needed by the forensic community of India for the identification of drugs and forensic interest compounds, poisonous plants especially those that are known to cause fatalities in humans from time to time.

The aim of the study can therefore be summarized as follows:

1. The unambiguous identification of those Forensic interest compounds previously detected at the Laboratory or that has been requested by the police authority, focusing on newly invented pesticides, drugs and plant Toxins.
2. Development of qualitative extraction procedures that would extract all the compounds of forensic interest. This is typically not a single extraction procedure but might be directed at Classes of compounds (alkaloids, terpenoids or cardiac glycosides, insecticides, herbicides, drugs).
3. Development of chromatographic and detection techniques based on the compounds of forensic Interest and at the typical concentration in which these compounds would be present in the forensic samples.
4. The application (if possible) of the mentioned extraction and detection procedures to forensic samples (crime scene exhibits, viscera, and blood, urine and stomach wash samples). If this is not possible, the above-mentioned procedures will be evaluated within a forensic matrix.
5. Quantification of forensically relevant compounds, as well as the determination of LOD (limit of detection) and LOQ (depending on availability of reference standards
6. Identification of those pesticides becomes essential to protect the ecology/mankind from the adverse effect of pesticide residues and forensic toxicology and criminology purposes.

1.2Forensic science:

The field of forensic science has come a very long way since its recorded beginnings in the 700s, when the Chinese used fingerprints to establish the identity of documents and clay sculptures. This field is one of the few areas of law enforcement where science, technology and crime solving meet. This combination supports the Theory of Transfer: "When two objects meet, some evidence of that meeting can later be found and verified".

A few significant advances occurred in the years prior to 1800. In 1248, a book His Duan Yu (The Washing Away of Wrongs) published by the Chinese, describing how to distinguish drowning from strangulation. It was the first recorded application of medical knowledge to the solving of crime. Paracelsus (1493 – 1541) introduced the use of drugs made from minerals. [2] On the subject of poisons, Paracelsus said "All substances are poisons: there is none which is not a poison. The right dose differentiates a poison from a remedy."[3] In 1609, the first treatise on systematic document examination was published in France. In 1784, one of the first documented uses of physical matching saw an Englishman convicted of murder based on the torn edge of a wad of newspaper in a pistol that matched a piece remaining in his pocket.[4]

Forensic science is a scientific method of gathering and examining evidence. Crimes are solved by the systematic evaluation of the crime scene followed by the gathering of fingerprints, palm prints, footprints, and any physical pieces of evidence that could prove useful in the investigation. This is followed by a pathological examination during which tooth bite prints, physical scars or wounds are examined and recorded, followed by a detailed autopsy of the deceased leading to the collection of various biological samples like blood, urine, hair and other body tissues.

Cyber and psychological profiling samples as well as ballistics techniques are often used to identify criminals or criminal acts, but all of the forensic samples or techniques mentioned before have one thing in common –

they all need sophisticated instrumentation and highly skilled staff.

Forensic science laboratories provide scientific aids to the police investigating officers in crime investigation, pertaining to crimes registered under various acts of Indian penal code and criminal procedure code etc. Forensic science laboratories are multidisciplinary institution doing highly specialized and sophisticated analytical work.

The indispensable analytical reports issued by the forensic science laboratories help the police and the judiciary in the detection of crime and administration of criminal justice by providing objective scientific evidence against the guilty or at times clearing the innocent.

1.3Forensic Toxicology:

Forensic Toxicology is the fundamental science of poisons. A poison is generally considered to be any substance that can cause severe injury or death as a result of a physicochemical interaction with living tissue. However, all substances are potential poisons since all of them can cause injury or death following excessive exposure [5]

Forensic Toxicology is an interdisciplinary scientific branch, which studies toxicological aspects of biology (toxicokinetic, toxicodynamic, experimental and clinical toxicology) and chemical sciences (chemical structure and analysis of poisons) Toxicological chemistry is a science, which works out new and develops present methods of detection and determination of poisonous substances in various objects, create theoretical fundamentals of these methods.[6]

Toxicology or the science of poisons is a fairly well-defined and sharply deman cated one. In practice this subject is divided into clinical and chemical toxicology. The forensic toxicologist is concerned with analysis from both the living and the dead. He has to find out the presence of any toxicologically

significant substance that may have contributed to the illness or death of the subject. Thus, cattle cases of a wide variety of areas of interest such as maliciously administered poisons or noxious substances with intent to murder, injury, aggrieve or annoy or perhaps with-intent to procure an abortion the investigations of sudden deaths of all categories to assist police are sent to the forensic toxicologist. Forensic toxicology is a multidisciplinary field involving the detection and interpretation of the presence of drugs and other potentially toxic compounds, chemical compounds in bodily tissues and fluids. Forensic toxicology covers all the compounds which shown in fig.1.

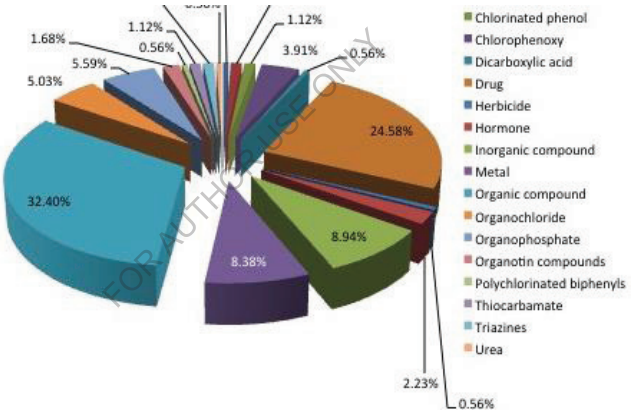


FIGURE 1: CHEMICAL COMPOUNDS RELATED IN FORENSIC TOXICOLOGY

In our country the pattern of poisons which have been ingested has changed, the older common poisons like opium, arsenic, Oleander, Datura etc. have been re-, placed by insecticides, barbiturates, alcohol etc. In this area thecases of organochlorine ~insecticides such as endrin, gammaxene, DDT have become very common. The definition of poison, types of poison as described below.

1.4Definition ofpoison:

A poison is a substance which, when administered, inhaled or ingested, capable of acting deleteriously on the human body. Thus, there are really no limits, between a medicine and a poison, for a medicine in a toxic dose is a poison and a poison in a small dose maybe a medicine means, it depends on dose/quantity only. In law, the real difference between a medicine and a poison is the intent with which itis given. If the substance is given with the intention to save life, it is a medicine but if it is given with the intention to cause bodily harm, it is a poison

A poison is a substance capable of producing adverse effects on an individual under appropriate conditions. The term "substance" is almost always synonymous with "chemical" and includes drugs, vitamins, pesticides, pollutants, and proteins. Even radiation is a toxic substance. Though not usually considered to be a "chemical," most radiations are generated from radioisotopes, which are chemicals. The term "adverse effects" above refers to the injury, such as structural damage to tissues. "Appropriate conditions" refers to the dosage of the substance that is sufficient to cause these adverse effects. The dose concept is important because according to it even a substance as innocuous as water is poisonous. [7]if too much is ingested. Whether a drug acts as a therapy or as a poison depends on the dose.

It is fact that virtually any substances can be harmful a high concentration- as Paracelsus (1493-1541), the father of toxicology said in the sixteenth century, “Everything is poison, there is poison in everything, only the dose makes a thing not a poison”

1.5 Classification of poison:

Poisons are of such diverse natures that they are classified by origin, physical form, chemical nature, chemical activity, target site, or use. [7]

1.5.1 Classification based onorigin:

Poisons are microbial, plant, animal, or synthetic origin.

Microscopic organisms such as bacteria and fungi produce microbial poisons. Botulinus toxin, for example, is produced by the bacterium Clostridium botulinum and is capable of inducing weakness and paralysis when present in under processed, nonacidic canned foods or in other foods containing the spores. An example of a plant toxin is the belladonna alkaloid hyoscyamine, which is found in belladonna (Atropa belladonna) and jimsonweed (Datura stramonium).

Animal poisons are usually transferred through the bites and stings of venomous terrestrial or marine animals, the former group including poisonous snakes, scorpions, spiders, and ants, and the latter group including sea snakes, stingrays, and jellyfish.

Synthetic toxins are responsible for most poisonings. "Synthetic" refers to chemicals manufactured by chemists, such as drugs and pesticides, as well as chemicals purified from natural sources, such as metals from ores and solvents from petroleum. Synthetic toxins include pesticides, household cleaners, cosmetics, pharmaceuticals, and hydrocarbons.

1.5.2 Classification based on physicalform: -

The physical form of chemicals – solid, liquid, gas, vapour, or aerosol – influences the exposure and absorbability.

Solids are generally not well absorbed into the blood; they must be dissolved in the aqueous liquid lining the intestinal tract if ingested or the respiratory tract if inhaled. Solids dissolve at different rates in fluids, however. For example, compared with lead sulphate granules, granules of lead are practically non-toxic when ingested, because elemental lead is essentially insoluble in water, while lead sulphate is slightly soluble and absorbable. Even different-sized granules of the same chemical can vary in their relative toxicities because of the differences in dissolution rates. For example, arsenic trioxide is more toxic in the form of smaller granules than is the same mass of larger granules because the smaller granules dissolvefaster.

A poison in a liquid form can be absorbed by ingestion or by inhalation or through theskin.Poisonsthataregasesatroomtemperature(e.g.,carbonmonoxide)areabsorbed mainly by inhalation, as are vapours, which are the gas phase of substances that are liquids at room temperature and atmospheric pressure (e.g., benzene). Because organic liquids are more volatile than inorganic liquids, inhalation of organic vapours is more common. Although vapours are generally absorbed in the lungs, some vapours that are highly soluble in lipids (e.g., furfural) are also absorbed through theskin.

Aerosols are solid or liquid particles small enough to remain suspended in air for a few minutes. Fibres and dust are solid aerosols. Aerosol exposures occur when aerosols are deposited on the skin or inhaled. Aerosol toxicity is usually higher in the lungs than on the skin. An example of a toxic fibre is asbestos, which can cause a rare form of lung cancer (mesothelioma).

Many liquid poisons can exist as liquid aerosols, although highly volatile liquids, such as benzene, seldom exist as aerosols. A moderately volatile liquid poison can exist as both an aerosol and as a vapour. Airborne liquid chemicals of low volatility exist only as aerosols.

1.5.3 Classification based on chemicalnature: -

Poisons can be classified according to whether the chemical is metallic versus non- metallic, organic versus inorganic, or acidic versus alkaline. Metallic poisons are often eliminated from the body slowly and accumulate to a greater extent than non-metallic poisons and thus are more likely to cause toxicity during chronic exposure.

Organic chemicals are more soluble in lipids and therefore can usually pass through the lipid-rich cell membranes more readily than can inorganic chemicals. As a result, organic chemicals are generally absorbed more extensively than inorganicchemicals. Classification based on acidity is useful because, while both acids and alkalis are corrosive to the eyes, skin, and intestinal tract, alkalis generally penetrate the tissue more deeply than acids and

tend to cause more severe tissue damage.

1.5.4 **Classification based on chemicalactivity: -**

Electrophilic (electron-loving) chemicals attack the nucleophilic (nucleus-loving) sites of the cells' macromolecules, such as deoxyribonucleic acid (DNA), producing mutations, cancers, and malformations.

Poisons also may be grouped according to their ability to mimic the structure of certain important molecules in the cell. They substitute for the cells' molecules in chemical reactions, disrupting important cellular functions. Methotrexate, for example, disrupts the synthesis of DNA and ribonucleic acid (RNA).

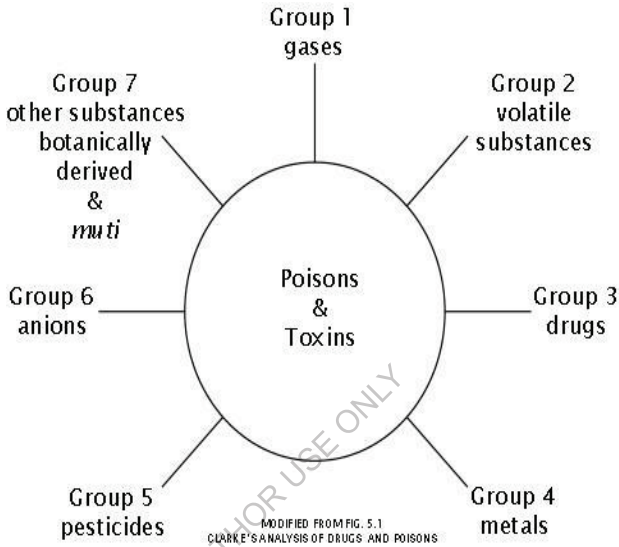
1.6**Other classifications ofpoisons:**

Unlike the classifications described above, there is usually no predictive value in classification by target sites or by uses. Such classifications are done, however, to systematically categorize the numerous known poisons. Target sites include the nervous system, the cardiovascular system, the reproductive system, the immune system, and the lungs, liver, and kidneys. Poisons are classified by such uses as pesticides, household products, pharmaceuticals, organic solvents, drugs of abuse, or industrial chemicals.

1.7**Forensic-chemical classification ofpoison: -**

Forensic toxicology demands an overall analytical system designed to exclude or indicate the presence of any poison in each of the chemical groups shown in Figure 2.1.[8] Most of the numerous screening procedures reported in the literature are too limited to permit a confident negative report. All too often these screening procedures are class/group directed, for example drug oriented, even though many criminal poisonings result from compounds other than drugs [5].

Toxicological chemistry describes all toxic substances in accordance to their isolationtechniquefromobjectsofinvestigation(internalorgansofcorpses,humanOrganism.



1.8Classification of Poisoning: -

The detection of a drug or poison is the most difficult part of the analytical toxicology process, as the nature of the poison is seldom known. Screening tests for any possible drugs or poisons should be used where there is no information available pertaining to the identity of the drug or poison. General screening methods are usually more flexible than special methods and can therefore be applied to a wide variety of materials. They are essential for the investigation of unknown poisonings and have some advantages even when the toxic agent is known or suspected. The deaths investigated by toxicologists include deaths from drug abuse, accidental poisoning, Intentional poisoning and homicidal poisoning.

1.8.1 Accidentalpoisoning: -

Most of the accidental poisonings occur in the home. The main cause of accidental poisoning in children occur due to the carelessness of adults who do not store poisonous compounds such as prescription drugs, detergents,

pesticides and household cleaners in such a way that they cannot be reached by children who are generally curious and adventurous in nature. Adult poisoning occurs usually because of mislabelling of products, which are not stored in their original containers. In industry, accidental poisoning is usually the result of carelessness.[6] Another agent that commonly leads to accidental poisoning is carbon monoxide, which is quite frequently generated by fossil fuel devices that is used in enclosed spaces without adequate ventilation. A few examples of such devices would be primus (paraffin) stoves and coal fires that are commonly made within informal dwellings during cold winter spells.

1.8.2 Suicidalpoisoning: -

In suicide, poisoning is a common manner of death. Suicidal poisoning, as already indicated, is of much bigger dimensions being three-fourth of the total number of poisoning cases. All suicides are resorted to as an escape from a situation, arising out of the multiplicity of the factors including the individuals own temperament and emotional stability, his social and economic security, as well as the pattern and intensity of his integration into society.

The most common suicidal agent is pesticides. While cyanide, arsenic and other well-known poisons may occasionally be used as suicidal agents, most deaths result from prescription drugs and pesticides. Nowadays, most suicidal poisonings involve multiple drug ingestion. By analysing the gastric and intestinal contents, blood, urine and the major organs of the body, the toxicologist can establish beyond doubt that the deceased could not have taken such a do accidentally[6]. In India, a new trend has developed among the Indian to commit suicide by the ingestion of a narcotic drugs. The use of plants or plant-based material to commit suicide has not been reported, largely due to uncoordinated collection of forensic and toxicology data in Maharashtra.

1.8.3 Homicidalpoisoning: -

Accidental and suicidal poisonings are common today, but murder by poison is rare in the first world countries. However, in third world countries

such as in Africa and the Eastern Pacific rim, murder by poison is not uncommon. To determine that a person died because of homicidal poisoning is one of the most difficult types of investigation. The general evidence of poisoning is obtained from knowledge of the symptoms displayed by the decedent before death, the post-mortem examination of the body by the pathologist and the isolation and identification of the poison by the toxicologist. Murder by poison most commonly occur within the home and the physician will seldom suspect a bereaved family member. Also, there are rarely any symptoms of poisoning that cannot equally well be caused by disease. The pathologist can recognise the effects of certain poisons at autopsy, but most poisons do not produce observable changes in body tissue and in most cases, toxicological analysis produces the evidence for murder by poison.[6]

Another cause is the poor evaluation of the crime scene, the lack of knowledge as to which samples should be taken and submitted for analysis, and a general unwillingness of people to get involved by supplying crucial information. Poisoning due to plant material (herbal poisoning) is also poorly reported, partly because of the unwillingness of people to admit using traditional medicine derived from plant material, but also because of the fear that the cultural heritage of the people will be "stolen" and commercialised[9].

The modern forensic toxicologist utilises a variety of analytical instrumentation and techniques to carry out his or her mission. Generally, the most difficult part of any toxicology analysis is the process of isolating the toxic substance from the biological matrix. This must be accomplished in a manner that provides a concentrated sample that contains the compound(s) of interest but is relatively free of interference by naturally occurring substances.

Once the compound is isolated, purified and concentrated, identification and quantification become possible[10].

In order to provide the pathologist or investigating officer with sound information as to the state of intoxication or poisoning, degree of impairment

or contribution to a fatality, the toxicologist must be informed as to the nature and circumstances of the intoxication as well as the quantity or concentration of the substance involved.[6]

The procedure utilised in a typical toxicology section of a laboratory may include preliminary examination of materials following its extraction and isolation by using chemical spot tests, which by colour production may indicate the presence of a type of drug or poison. More sophisticated techniques includethin-layer chromatography (TLC), ultraviolet spectroscopy (UV); infrared spectroscopy (IR); high performance liquid chromatography (HPLC) and gas chromatography (GC). The application of mass spectrometry (MS) is now commonly utilised for positive identification of drugs and toxic compound.

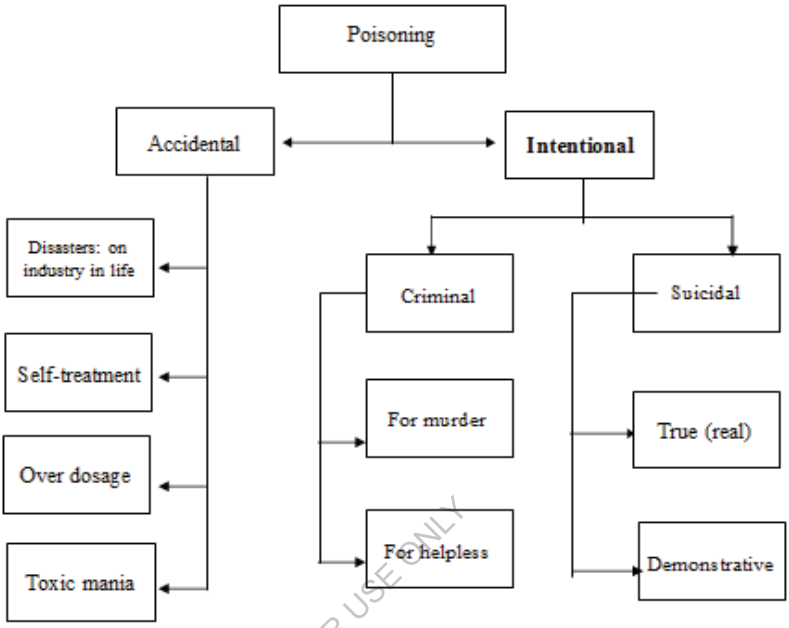


FIGURE 2: FLOW DIAGRAM OF CLASSIFICATION OF POISONING

1.8.4 Poisoning due to over dose of drug: -

1.8.4.1 Definition of drug:

1. .Natural or synthetic substance which (when taken into a living body) affects its functioning or structure, and is used in the diagnosis, mitigation, treatment, or prevention of a disease or relief of discomfort. Also called legal drug or medicine. A legal or medicinal drug (such as amphetamines), however, can be harmful and addictive if misused.
2. .Habit forming stimulant or narcotic substance (such as alcohol, cannabis, nicotine, or a derivative of cocoa or poppy) which produces a state of arousal, contentment, or euphoria. Continued or excessive

use (called drug abuse or substance abuse) of such substances causes addiction or dependence. [11]

FIGURE 3: VARIOUS FORMS OF DRUG

Thereafter any attempt to discontinue their use results in specific reactions (called withdrawal symptoms) such as sweating, vomiting, and tremors which cease when theuse is resumed. Also called illegal drug where its production and/or use is prohibited. Whether a substance is legal or illegal, however, may have nothing to do with its potential for addiction or harm: alcohol and nicotine, both addictive and harmful, are legal in most countries because they generate substantial employment or government revenuethroughtaxes.

1.8.4.2Types of Drug: -

A drug is any kind of medicine or chemical that changes how your body

or brain functions. There are legal drugs, which you can buy over the counter or get from a health professional, and illegal (or 'street') drugs. Drugs can be categorized based upon their effect on user. Most illegal drugs

fall into one of seven drug typeeach with its own set of characteristics, effects and danger. Categories includes:stimulants, depressants, hallucinogens, dissociative, opioids, inhalants, cannabis.[14]

- **Stimulants**: Stimulants often come in pill form but are also consumed via snorting or even as food and drink. For example, caffeine is found in many beverages,andcocaineis a powder that is snorted. *Examples of stimulants include: Adderall, Ritalin Synthetic Marijuana,Cocaine, Methamphetamine, Ecstasy, Caffeine*

- **Depressants:**Doctors prescribe some depressants for anxiety, insomnia, obsessive-compulsive disorder and other medical issues that prevent the sufferer from fully relaxing. These drugs often offer a sedative experience to users, making them a tempting choice for teens who wish to escape everyday stresses. *Examples of depressants include: RohypnolBarbiturates, Xanax, Valium,**Benzodiazepines.**Alcohol acts as a depressant.*
- **Hallucinogens:** Hallucinogens work by disrupting communication within the brain. Users report intense, rapidly shifting emotions and perceptions of things that aren't really there. For example, a hallucinogen user might believe that they see a person speaking to them — when that person does not even exist. Hallucinogens come in many forms, which can be smoked, eaten, ingested. *Examples of Hallucinogens include: LSD Psilocybin Salvia Peyote*
- **Dissociative:**These drugs work by interfering with the brain's receptors for the chemical glutamate, which plays a significant role in cognition, emotionality and pain perception. Dissociative can be taken as liquids, powders, solids or gases.*The drugs include: Ketamine DX(Dextromethorphan)PCP (phencyclidine)*
- **Opioids:**Opioids can be smoked, eaten, drank, injected or taken as pills.

Examples of opioids include: Heroin Morphine Hydrocodone Opium Vicodin Oxycontin Percocet codeine

- **Inhalants**: Mostly made up of everyday household items, these drugs cause brief feelings of euphoria. As the name suggests, inhalants are always inhaled as gases or fumes. The "highs" slightly differ from inhalant to inhalant, but most abusers are willing to huff whatever inhalant they can acquire.Examples of inhalants include: Fumes of markers, paint, paint thinner, gasoline and glue Nitrousoxide Aerosolsprays Room deodorizers
- **Cannabis: It** can be smoked, vaporized, and even eaten, if the THC is first rendered from the plant matter.Examples of cannabis include: Marijuana leaves Hashish Hash oil Cannabis-based medicines, such as Sativex

Drug abuse, the non-medical use of drugs or other chemicals for the purpose of changing mood or inducing euphoria, is the source of many poisonings. Drug abuse may involve the use of illicit drugs such as heroin or phencyclidine, the use of restricted or controlled drugs such as cocaine, barbiturates and amphetamine, or use of chemicals in a manner contrary to the intended purpose – such as inhaling solvents and aerosol products [6]. In a broader sense, drug abuse may also include the excessive use of legal substances such as alcohol and prescription drugs[6].

Alcohol is a self-limiting poison as people usually lose consciousness before a lethal dose isingested and therefore overdose deaths due to the ingestion of excessive quantities of alcohol are uncommon. However, numerous accidental deaths occur from the concurrent ingestion of potent prescription drugs and alcohol [6]. It must be noted that many drugs were initially derived from plant material (crude morphine from Papaver somniferum L., cocaine obtained from coca, the dried leaves of Erythroxylum coca and other species of Erythroxylum) or are plant products chemically modified to produce more potent drugs. (morphine acetylated with acetic anhydride to produce heroin)

1.9Pesticides: -

1.9.1 Definition of Pesticides: -

The Food and Agriculture Organization (FAO) has defined pesticide as:

"any substance or mixture of substances intended for preventing, destroying, or controlling any pest, including vectors of human or animal disease, unwanted species of plants or animals, causing harm during or otherwise interfering with the production, processing, storage, transport, or marketing of food, agricultural commodities, wood and wood products or animal feedstuffs, or substances that may be administered to animals for the control of insects, arachnids, or other pests in or on their bodies."

Pesticides are chemical substances that are meant to kill pests. In general, a pesticide is a chemical or a biological agent such as a virus,

bacterium, antimicrobial, or disinfectant that deters, incapacitates, kills, pests .[10-12]

FIGURE 4:PESTICIDES

This use of pesticides is so common that the term pesticide is often treated as synonymous with plant protection product. It is commonly used to eliminate or control a variety of agricultural pests that can damage crops and livestock and reduce farm productivity. The most commonly applied pesticides are insecticides to kill insects, herbicides to kill weeds, rodenticides to kill rodents, and fungicides to control fungi, mild, and mildew.[13]

According to its chemical structure, pesticides are classified into

different families, ranging from organochlorine and organophosphorus compounds to inorganic compounds. In this thesis, we refer only some families of pesticides relevant for the damage they cause to human health and high demand for its use. The most common way to classify them based on their chemical structure is split into four main groups: Organochlorine Stable compounds are too persistent in the environment.

Types of Pesticides: These are grouped according to the types of pests which they kill:[15-18]

1.9.2Grouped by Types of Pests They Kill,

- **Insecticides**: used for killing insects. e.g. Sevin
- **Herbicides**: used for killing weeds or herbs. e.g., Gram Oxone

- **Rodenticides**: used for killing rodents (rat, mice) e.g. Klerat
- **Bactericides**: used to killing Bacteria
- **Fungicides:** used for killing fungi. e.g. mankocide
- **Larvicides**: used to killing larvae

1.9.3 Based on how biodegradable they are:

Pesticide can also be considered as Biodegradable: The biodegradable kind is those which can be broken down by microbes and other living beings into harmless compounds. Persistent: While the persistent ones are those which may take months or years to break down. Another way to classify these is to consider those that are chemical forms or are derived from a common source or production method.

1.9.4 Chemically-related pesticides:

1.9.4.1Organophosphate Pesticides: -

An organophosphate (sometimes abbreviated as OP) is the general name foresters of phosphoric acid. Phosphates are probably the most pervasive organophosphorus compounds. Organophosphates are also the basis of many insecticides, herbicides, and nerve gases. Organophosphates are widely used as

solvents, plasticizers, and EP additives.[12]

The First OPs were synthesised in the 19th century, but they only started to be used widely in the 1930s. The German chemist Gerhard Schrader synthesised many commercial OPs of which parathion (Figure 2) is still used as a common pesticide (Costa, 2006). At the beginning of the Second World War the development of OP substances switched to highly toxic compounds employed as nerve warfare agents, e.g. sarin, soman and tabun (Figure 5). After the War, in the 40's and 50's, the study of OPs was again oriented towards the development of less toxic compounds (Gupta, 2006). However, OP pesticide usage increased rapidly in the 70's, when the application of organochlorine pesticides such as DDT was prohibited because of their long-life persistence in the environment[19.]

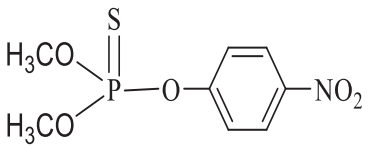
OPs are esters of phosphoric acid and its derivates. The general chemical structure of an organophosphate (Figure 4) comprises a central phosphorus atom (P) and the characteristic phosphoric (P=O) or thiophosphoric (P=S) bond. The symbol X represents the leaving group, which is replaced (by nucleophilic substitution) by the oxygen of serine in the AcHE active site. The rate of AcHE inhibition depends on the leaving group; higher tendency of leaving results in higher affinity of the inhibitor to the enzyme.

FIGURE 5: GENERAL STRUCTURE OF OPS

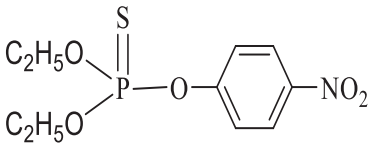
X represents the leaving group, R1 and R2 the side, usually alkoxy, group (adapted from Hreljac, 2009). In very toxic warfare agents the leaving group contains fluorine (F), which has high tendency to hydrolysis and thus

extremely high AcHE inhibition. In less toxic OPs the leaving group usually contains alkyl or aryl groups. Side groups R1 and R2 are usually alkoxy groups. The active configuration of OP, which binds to the AcHE active site, is an oxo no structure, with the central phosphorus linked to the oxygen atom (Figure 2b). The majority of novel OP pesticides possess the thion, P=S, linkage (Figure 2a). In this case, metabolic activation with CYP enzymes (cytochrome P450) must first metabolize the thiono to an oxo no group – only then can the OP act as an AcHE inhibitor (Gupta, 2006). [20]

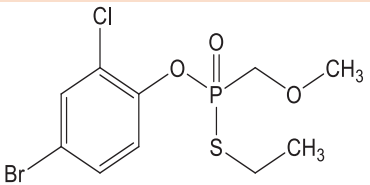
Following Organophosphate pesticides are mostly highlighted pesticides in forensic interest.



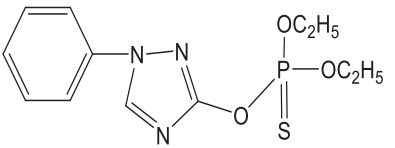
Methyl parathion



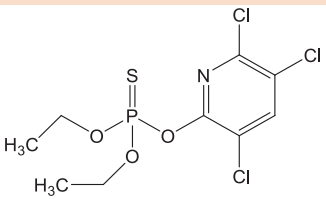
Ethyl parathion



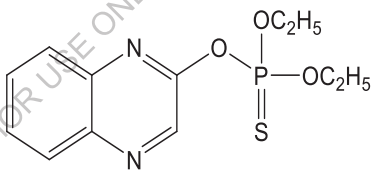
Profenophos



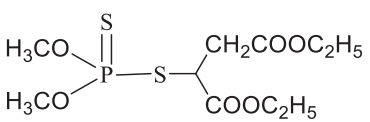
Triazophos



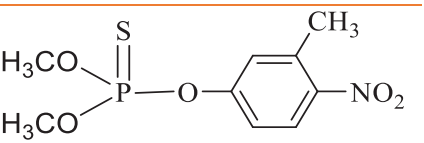
Chlorpyriphos



Quinalphos (Ekalux) ·



Malathion



Fenitrothion

FIGURE6:CHEMICAL STRUCTURE OF ORGANOPHOSPHORUS AND TRIPHOSPHOROUS INSECTICIDES

Organophosphate pesticides are used extensively worldwide and poisoning by these agents, particularly in developing nations, is a serious public health problem. Various method haven been reported in literature for the

detection and determination of organophosphorus insecticide. These include gas chromatography, spectrophotometer, high performance liquid chromatography, fluorometry, etc. But all these methods are reported for pure compound or for formulated products. In case of biological material due to highly susceptible impurities clean up procedure is required. Here TLC and HPTLC methods are used for detection of organophosphorus compound from biological materials.Many organophosphates are highly toxic to aquatic organisms.[22]

1.9.4.2 Carbamate Pesticides: -

Investigations of chemicals that exert an anticholinesterase action on the nervous system similar to organophosphates led in the 1950s to the development of the carbamate insecticides. The carbamate insecticides are derivatives of carbamic acid (as the OPC are derivatives of phosphoric acid). And like the OPC, their mode of action is that of inhibiting the vital enzyme cholinesterase. These compounds have general formula as Substitution of sulphur for oxygen also occurs, but such compounds generally have low insecticidal activity. The first successful carbamate insecticide, carbaryl (Sevin), was introduced in 1956. More of it has been used world-wide than all

the remaining carbamates combined. Two distinct qualities have made it the most popular carbamate: it's very low mammalian oral and dermal toxicity and an exceptionally broad spectrum of insect control.

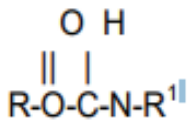
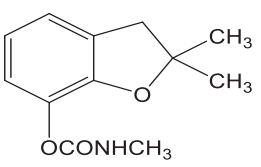
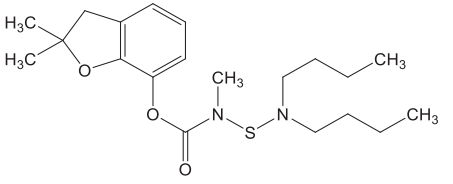


FIGURE 7:CHEMICAL STRUCTURE OF CARBAMATE PESTICIDES

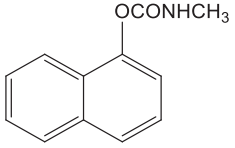
Carbamate insecticides are derivatives of carbamic acid, HOC(O)NH2. They have the general formula shown above where R is an alcohol, oxime or phenol and R1 is hydrogen or a methyl group. O H ‖ | R-O-C-N-R1 Carbamates vary in their spectrum of activity, mammalian toxicity and persistence. They are relatively unstable compounds that break down in the environment within weeks or months. Carbamates are commonly used as surface sprays or baits in the control of household pest .[21.]

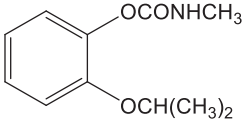
Carbamates are esters of N-methyl carbamic acid. Aldicarb, carbaryl, propoxur, ox amyl and terbucarb are carbamates. Although these pesticides differ chemically, they act similarly[23]. When applied to crops or directly to the soil as systemic insecticides, organophosphates and carbamates generally persist from only a few hours to several months. However, they have been fatal to large numbers of birds on turf and in agriculture, and negatively impacted breeding success in birds. Many organophosphates are highly toxic to aquatic organisms. Similar to the organophosphorus pesticides, the carbamate pesticides also affect the nervous system by disrupting an enzyme that regulates the neurotransmitter. However, the enzyme effects are usually reversible.[24]





Carbosulfan **Carbofuran (Furadan)**





Carbaryl((Sevin)	Propoxur (Baygon)

FIGURE 8:CHEMICAL STRUCTURE OF CARBAMATE PESTICIDES

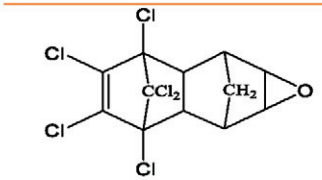
Carbamates are used as either dusts or sprays. They may be absorbed through the skin as well as by ingestion and inhalation. Among the various analytical methods used for the detection and determination of carbamate insecticides, TLC & HPTLC are the most useful and most applicable method for these insecticides in biological material and non-biological materials in forensic toxicology.

1.9.4.3 OrganochlorinePesticides: -

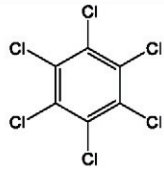
At the beginning of the twentieth century, early investigations with chemical pesticides led to the widespread use of inorganic compounds within agriculture containing elements such as sulphur, arsenic, mercury, lead and other metals. Some natural products, such as pyrethrum, were also known to be effective pesticides at this time but were considered too expensive for widespread use. Between the world wars, the development of the chlor-alkali industry provided the raw material for the mass production of synthetic chlorinated organic molecules. With the increasing desire for selective

biocides within agriculture, and the emerging chemical technologies, the hunt for synthetic pesticides was on. An early chlorinated phenoxy acid herbicide (2,4-D) was first discovered in 1932. Although this compound rapidly breaks down in the environment, the seed fungicide hexachlorobenzene (HCB), introduced in 1933, was found to be far more persistent. The structurally similar insecticide hexachlorocyclohexane or BHC.[25]

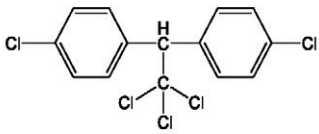
The chemical structure of organochlorines is diverse but they all contain chlorine, which paces them in a larger class of compounds called chlorinated hydrocarbons·.



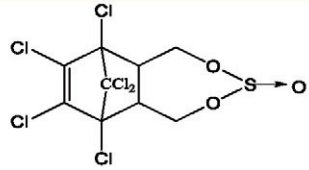
Endrin



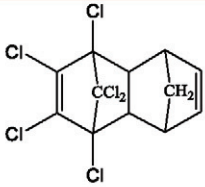
BHC



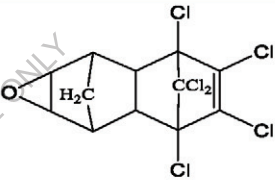
DDT



Endosulfan



Aldrin



Dieldrin

FIGURE 9:CHEMICAL STRUCTURE OF ORGANOCHLORINE PESTICIDES

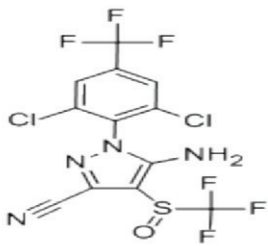


FIGURE 10:CHEMICAL STRUCTURE OF FIPRONIL

Organochlorine pesticides are chlorinated hydrocarbons used extensively from the 1940s through the 1960s in agriculture and mosquito

control. Representative compounds in this group include DDT, methoxychlor, dieldrin, chlordane, toxaphene, mirex, kepone, lindane, and benzene hexachloride. As neurotoxicants, many organochlorine pesticides were banned in the United States, although a few are still registered for use in this country.

People can be exposed to organochlorine pesticides through accidental inhalation exposure if you are in an area where they were recently applied.[26.] The chemicals can also be ingested in fish, dairy products, and other fatty foods that are contaminated.[27] They were commonly used earlier, but now many countries have been removed Organochlorine insecticides from their market due to their health and environmental effects and their persistence (e.g., DDT, chlordane, and toxaphene). In the last few years, several studies have been published based on the development of more precise, cheaper and faster analytical procedures.[28]

Analytical chemistry has witnessed a significant improvement with the establishment of new methodologies by the use of more sensible instrumental techniques that use small quantities of samples, such as mass spectrometry (MS), Fourier Transform Infrared Spectroscopy (FTIR) and Ultraviolet-Visible Spectrometry (UV-Vis) and Raman Spectrometry (RAMAN) But these techniques required skilled man power. TLC and HPTLC techniques are best

option for detection of pesticides from biological and non-biological materials in forensic science laboratories.[29]

1.9.4.4 PyrethroidPesticides: -

The historical development of the synthetic pesticides called pyrethroids is based on the pyrethrin's, which are derived from chrysanthemums. Pyrethrum is a compound extracted from Chrysanthemum flowers Pyrethrin's are a "natural" environmental product that is of low toxicity to mammals. They are highly photolabile and degrade quickly in sunlight, and the cost of reapplying them has limited their widespread agricultural use.

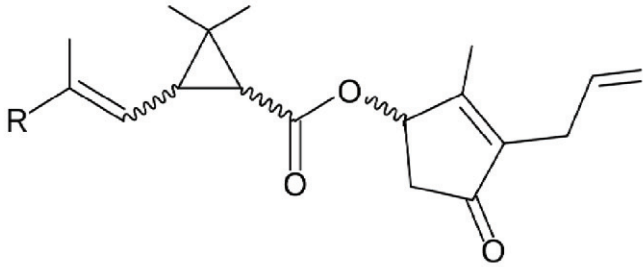


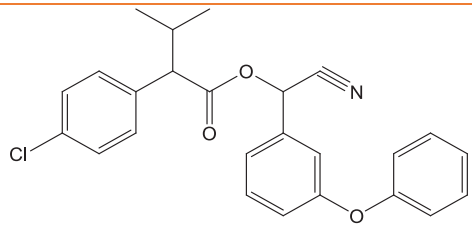
FIGURE 11:CHEMICAL STRUCTURE OF PYRETHROID

Pyrethroids have been synthesized to be similar to pyrethrin yet more stable in the environment. Evidence suggests that they have a very large margin of safety when used as directed by the label (Aldridge, 1990; Chen et al., 1991; Snodgrass, 1992). Commercial pyrethroid products commonly use petroleum distillates as carriers. Some commercial products also contain OP or carbamate insecticides because the rapid paralytic effect of pyrethrin on insects ("quick knockdown") is not always lethal (Cheremisinoff and King, 1994). Pyrethroids are synthetic variations of naturally found pyrethrums.

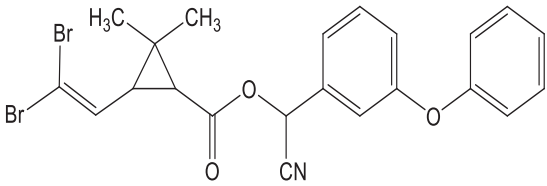
The advantage to creating pyrethrin's in the laboratory is that the compounds tend to be more potent and last longer in the environment, two properties desired for pest control.[27] Pyrethroids are formulated as emulsifiable concentrates, wet table powders, granules, and concentrates for ULV application. PERMETHRIN General Information Permethrin is a broad-

spectrum pyrethroid insecticide. It is available in dust emulsifiable concentrates, smokes.

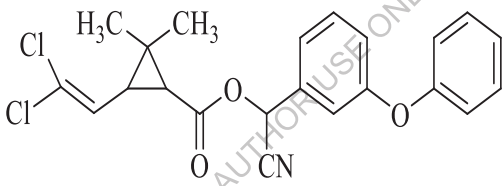
Pyrethroid pesticides are used in agriculture, mosquito control, lawn and garden care, and in veterinary care. Some representative pyrethroidsarepermethrin, resmethrin, Fenvalrate, cyfluthrin, Deltamethrin.



Fen valerate



Deltamethrin



Cypermethrin

FIGURE 12:CHEMICAL STRUCTURES OF PYRETHROIDS

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Chapter 1

Section C

Literature survey

The first global estimates of the extent of pesticide poisoning were published in 1990 by the World Health Organisation (WHO) (WHO, 1990). Based on extrapolations from limited data, it was estimated that 3 million cases of pesticide poisonings annually occurred worldwide, with 220,000 deaths; the majority of which are intentional (Konrad Sen, van der Hoek et al., 2003).[1,2,3] The WHO estimates, based on data from 2001, that 849,000 people die globally from self-harm each year (WHO, 2002). How many of these cases are

a result of poisoning with pesticides is not known. However, poisoning is the most common form of fatal self-harm, such as suicides, in rural Asia,

accounting for over 60% of all deaths (Somasundaram & Rajadurai, 1995; Phillips, Li et al., 2002; Joseph, Abraham et al., 2003) and is of far greater importance than hanging and other physical forms of self-harm. Furthermore, a review of poisoning studies reveals that pesticides are the most common way of self-poisoning in many rural areas and are associated with a high mortality rate (Eddleston, 2000). [4,]

A recent national survey, in the year 2000, in Bangladesh showed that 14% of all deaths (3971 of 28,998) of women between 10 and 50 years of age were due to self-poisoning; the majority of which used pesticides (Yusuf, Akhter et al., 2000)[5]. The problem is particularly severe in Sri Lanka (Berger, 1988; Van der Hoek, Konrad Sen et al., 1998),[6] where pesticide poisoning was the commonest cause of hospital death in six rural districts in 1995 (Srilanka, 1995). In many countries, the widespread availability of acutely toxic pesticides used in agriculture has made the selection of pesticides as the agents of choice for self-harm well known to both healthcare workers and public-health authorities (Nalin, 1973; Kasilo, Hobane et al., 1991[7]; Daisley & Hutchinson, 1998)[8]. According to the WHO, one million serious accidentals and two million suicidal poisonings due to insecticides occur worldwide every year, of which 200,000 patients die with most deaths occurring in developing countries.[8]

In India, organ compounds (OPCs) organophosphates and organ carbamates are the commonest pesticides used and due to their easy availability, there is widespread abuse of these compounds with suicidal intent Poisoning due to insecticides and pesticides has an important role in crimes all over the world. Forensic toxicologists are dealing with maximum number of cases' due to insecticides and pesticide poisoning involved in various types of crimes.

Pesticides also cause much hazard to the surrounding environments and other organisms. Since the analysis of insecticide residue possess an entirely

different type of problem for the toxicologists because these residues are present in extremely small quantity in heterogeneous materials including the biological materials. The importance of insecticide residue problem led to intensive search for analytical methods for accurate and rapid analysis was studied by Zweig1, the determination of the pesticide in various biological materials often faced with the problem of determining the minute quantity of insecticides mixed with large amount of extraneous material or intermixing. Qualitative and quantitative methods are required to be applied keeping in view the sensitivity and specificity of the methods on one hand and nature of type of pesticides on the other hand.

The technique of thin layer chromatography has made a strong impact on analytical toxicology. Extensive literature on both the qualitative and quantitative analysis of insecticides/pesticides is available.2 or spectrum a Thin Layer chromatography which is much sensitive and quicker than ordinary chromatography, is important technique in toxicology and being used in several laboratories for general screening of biological samples for alkaloids, pesticides and other drugs, Various reagents are reported in literature for the detection and determination of organophosphorous insecticides by TLC and HPTLC. Baumler and Hippstein have reported [9] the use of Palladium (II) chloride for the detection of organophosphorous insecticides

A large number of chromogenic reagents have been described in literature for identification of organo phosphorus and organo chlorine pesticides. But in all cases both groups of pesticides have been dealt separately. According to recent publication 33 successive spraying of plates with palladium chloride and diphenyl amine solutions can locate pesticides of both the groups on the same chromatoplate. The chloro-naphthalene group of pesticides can conveniently be detected by spraying the plates with 0.1 N

KMn04 solution made alkaline with sodium carbonate which is decolourised at the spot and the place becomes visible against a violet back ground.

Helvctica. Irving Sunshine et al [10] have published chromatographic data of a large number of commonly used drugs in seven different solvent systems.A.R. Sperling[11] had analysed a mixture of hallucinogens like L.S.D. and S.T.P, (2,5-Dimethoxy-4 methyl amphetamine) by thin layer chromatograph. Many methods of Thin Layer chromatography had been published for isolation of organo-phosphorus and organo-chlorine insecticides from blood and viscera.[12,13] Coutselinis and Dimopoulos [14] had described a single rapid technique for simultaneous extraction and separation of both organo-phosphorus and organo-chlorine insecticides.

A Modification in development of thin layer plates was introduced by KHO and Klein [15].in which sulphonamides and sulpha drugs were separated in a better way using several solvents successively in order of their decreasing polarity in the same direction on the same chromatoplate.

The literature survey reveals a large number of research activities are cantered in identification of organophosphorus, organochlorine, carbamate and pyrethroid insecticides. The available methods for qualitative detection of pesticides by thin layer chromatography have been re-evaluated in order to develop technique which could simultaneously detect major organo-chloro and organo-phosphorus pesticides by suitable chromogenic reagents on single TLC plate. Several other modifications of TLC like use of polyamide surface and detran gel surface [16] are yetto be utilised for toxicological work. The pesticides studied were dieldrin, aldrin, DDT, lindane, endrin, malathion, parathion, dimethoate and phosphamidon, The Chromatogram on silica gel or alumina plates were developed in first in respect of organo-chloro pesticides and then the same plates were treated for organo-phosphorus pesticides. The following combination of chromogenic reagents have offered good results out of a large869number of chromogenic reagents studied:

I) Zinc chloride and diphenylamine reagent for development of organo-chloro pesticides followed by either bromophenol blue-

silver nitrate-acetic acid reagent or para-nitro benzyl-pyridine and tetra ethylene pentaamine reagent for organo-phosphorus pesticides.

II) Silver nitrate and 2-phenoxy ethanol reagent for organo-chloro pesticides followed by fluorescein reagent to detect organo-phosphorus pesticides.

III) Ortho-dianisidine reagent for Organochloride pesticides followed by 2,6 dichloro-quinone-chlorimine or Bromophenol blue silver nitrate acetic acid reagent or p-nitro -benzyl pyridine and tetraethylene-pentamine reagent for organo-phosphorus group of pesticides

Sonnenfeld and Paulhavedeveloped[17] technique of organophosphorus insecticides dichlorophos, ethion or phorate, fenaulfothion, oxydemeton-methyl, phosmet, phospholan and trichlorfon have been appeared from each other on silica gel foils and detected with silver nitrate-UV reagent.

Kaur and Garghave studied[18]detection on the persistence of dimethoate and phosphamidon insecticides from soil and paper substrates by thin layer chromatography. Among the fifteen different solvent systems examined, it has been observed that dimethoate can be separated by using benzene: acetone (9:1) and phosphomidon by using cyc1ohexane: acetone: chloroform (70:25:5) followed by iodine fuming as visualizing aid. The analysis of these two compounds could be successfully performed even up to 8 weeks times from soil and paper containing these pesticides in small amounts and it remains therein.

Eddleston et al. have studied[19] poisoning with the S-Alkyl organo-phosphorous insecticides Profenophos and prothiofos. Compared with other commonly used OP insecticides, Profenophos and prothiofos are of moderately

severe toxicity, causing relatively delayed respiratory failure and death. There was no apparent response to oxime therapy. The lack of correlation between

red cell AcHE activity and clinical features suggests that this parameter may not always be a useful marker of synaptic AcHE activity and severity after OP pesticide poisoning.

Bahman Ebrahimi hasstudied[20]a new changeable bioreactor for detection of organophosphate in a flow-through system. A flow-through biosensor consisting of a fixed bed bioreactor was employed to detect the insecticide paraoxon. Based on the inhibition of organophosphorus insecticide to the enzymatic activity of acetylcholinesterase (AcHE), using paraoxon as a model compound, the condition for detection of the insecticide was optimized. The influence of enzyme loading on the packing surface was studied.

Martin *et al*. have reported eight OP insecticides have been separated and identified in water by silica gel HPTLC and detection by 2 methyl thiouridine spray reagent.[8].

Dhingra Vinod has studied[21] about the acephate is an organophosphorus insecticide of broad spectrum used in field crops. He described symptoms, post-mortem changes occur in a reported case of acephate insecticide and detection and identification of insecticide in visceral material by TLC.

. A sensitive spray reagent 4- (P-nitro benzyl) pyridine tetra ethylene-pentaamine has been reported by Zweig, for the detection of organophosphorus insecticide. Kawale and et al. have reported[22] mercury(I) nitrate reagent & Joglekar, et al.have reported[23]mercury (II) nitrate-diphenyl carbazone reagent, which were used for the detection of derivatives of barbituric acid were further utilized for detection of organophosphorus and organ thiophosphate insecticides. There is some exception such as corticosteroid, chloromycetin, etc.

Sharma & Boymal have worked[25] on environmental samples and have

been quantified on organophosphorus insecticides by densitometry on C-18 layer using N,2,6-tricholorobenzoquinonaimine spray reagent for detection

Betowski and Joneshave described[26] a high-performance liquid chromatography mass spectrometry method (HPLC-MS) for analysis of OP insecticides in soil. Patil *etal*. have reported a new chromogenic spray reagent for OP insecticide monocrotophos. Monocrotophos on alkaline hydrolysis yields dimethyl phosphoric acid and *N*-methyl acetoacedamide. The latter reacts with a chromogenic reagent chloranil[24] to form a red coloured spot.

Katkar and Barve have developed[27] spray reagent mercuric nitrate followed by potassium ferrocyanide for detection of organophosphorus insecticide, bluish colour spot was observed after keeping plate for about 5 min at room temp.

Patil and Shingare have developed[28] a spray reagent for selective detection of dichlorvos in biological materials by thin-layer chromatography. Dichlorvos in presence of moisture breaks down to trichloroacetaldehyde which in turn reacts with phenyl hydrazine hydrochloride to give a yellowish red colour. In acidic media the colour is intensified and consequently the sensitivity of detection increases. The reagent is selective for dichlorvos, other organophosphorus insecticides failed to give a coloured spot.

Patil and Garad have developed [29] another spray reagent for detection of monocrotophos from biological material. Monocrotophos on alkaline hydrolysis yields N-methyl acetoacedamide which further reacts with chloranil to give red colour complex. The active methylene group reacts with the chromogenic reagent chloranil to give red colour compound. Chloranil has been used for the detection of primary, secondary amine and phenol. Monocrotophos and phenol gives red and brown spot and hR_f from extract of viscera at hR_f 45.

Shano *et al*. have reported the use of phosphomolybdic acid (20 % w/v

inethanol) as a chromogenic spray reagent for the detection and identification of permethrin, cypermethrin and deltamethrin[12] whereas palladium chloride (0.5 % w/v) in (12 moldm -3HCL) has been described for the detection of

deltamethrin [13]and silver nitrate impregnated alumina G and irradiation with uv light [14] for detection of pyrethroid insecticides in general.

Mali et al. have developed[30] spray reagent for detection of dichlorvos and dimethoate using orcinol. The alkali hydrolysis product of dichlorvos and dimethoate reacts with orcinol produces a yellow fluorescent compound.

Shivhare has reported a spectrophotometric method[31] for the determination of methyl parathion residues in plant material and soil. The reaction is based on reduction of nitro group present in parathion methyl with Zn-HCL to form an amino group which is subsequently diazotized and coupled with guaiacol in alkaline medium to form a yellow coloured azo-dye, which show λ max at 470 nm. Other commonly found pesticides do not interfere.

Zoun et al. have studied[32] over 100 cholinesterase inhibiting pesticides have been separated by HPTLC and detected by spraying with bovine liver suspension followed by an appropriate ester and chromogenic reagent. The method was used to detect pesticides in biological and environmental samples and foods.

Sastry and Manala have described[33]three spectrophotometric method for the determination of carbaryl and propoxur in insecticidal formulation, water and grains, based on the formation of coloured species with p-aminophenol-N, N-dimethylphenanylene diamine dihydrochloride and 1-amino-3-napthol-4-sulphonic acid respectively under specific experimental conditions.

Bhatia J. has studied[34] thin layer chromatographic and spot test detection of carbosulfan, a carbamate pesticide by alkaline fast blue-B reagent. Carbosulfan on alkaline hydrolysis forms a phenolic product which further condenses with fast blue-B and forms orange coloured complex on silica gel-G TLC plate.

Coutselinist and Kentarchou have reported[35] an ethanolic diphenylamine reagent for the detection of organochlorine insecticide by TLC. Thielemann has

used[36]sodium hydroxide followed by methanolic thymol for the detection of chlorinated pesticides.

Makhubalo and Mainga have describeed[37]3,3',5,5'-tetramethylbenzidine reagent for detection of organochlorine pesticides. Sharma has further developed[38]a technique for six components of organochlorine insecticide mixture containing BHC, methoxychlor, heptachlor epoxide, dieldrin and aldrin have been successfully resolved on a C-18 chemically bonded RP layer by development with acetonitrile-water (75+25). Minimum sensitivity for visual detection and densitometric scanning ranged from 300-900 ng upon detection with o-toluidine reagent.

Carbaryl was hydrolysed to give 1-napthol which was determined with 4-aminoantipyrine $K_3(Fe\ (CN_6)$ at PH[10]. Phosphamidon on oxidation with iodine, HNO_3 or H_2O_2 produced phosphates and could be determined by titrating unreacted iodine with $Na_2S_2O_3$. Parathion has been determined by reducing it to a primary amine and determining the amine calorimetrically after diazotisation and coupling with N-1-naphthylethylene diamine.

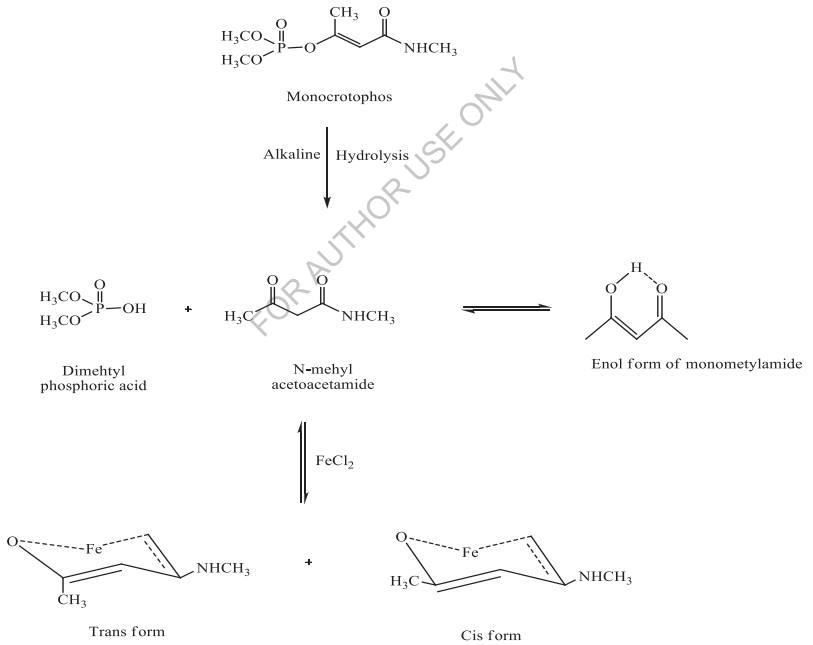
Akmal et al. have reported[39] a thin layer chromatographic method for detection of pyrethroid insecticides. These insecticides, on brominating and treatment with o-toluidine, yield an intensely blue product. 2.4-Dinitrophenylhydrazine and phosphomolybdate has also been used as a chromogenic reagent for thin layer chromatographic detection of synthetic pyrethroid insecticides in biological materials. Various other workers also studied various types of reagent for detection and identification of pyrethroid insecticide in food grain and water.[40-46]

Martin *et al*. have reported eight OP insecticides have been separated and identified in water by silica gel HPTLC and detection by 2 methyl thiouridine spray reagent.[47]

Krishna V. Kulkarni1, Dhananjay V. Mane,*[49] *et al* have reported a high-performance thin layer chromatographic method for detection and

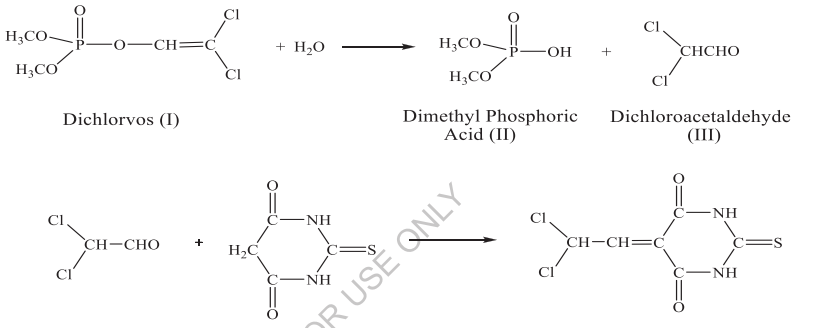
identification of Carbosulfan-carbamate pesticides. Alkaline hydrolysis of Carbosulfan yields the sodium salt of 2,3-dihydro-2,2-dimethylbenzofuran-7-ol, which forms a purple complex with potassium ferricyanide in the thiochromone reaction.

Kulkarni *et al.*[50] have studied a new chromogenic spray reagent for chromatographic detection & identification of Monocrotophos, an organophosphorus insecticide is described by HPTLC method. Monocrotophos on alkaline hydrolysis yields one molecule each of *O*, *O*-dimethyl phosphoric acid, and *N*-methyl acetoacedamide. After acidifying, *N*-methyl acetoacedamide gives enol form of monomethyl amide which reacts with ferric ions to yield purple colour complex.



Chandegaonkarand Maneetal.[51], have reported TLC detection of monocrotophos using vanillin. The alkali hydrolysis product of monocrotophos reacts with vanillin to produce a green colour compound.

Rane *et al*.[52] have reported a new chromogenic spray reagent 2-thiobarbituric acid for detection of dichlorvos in biological materials by using thin layer chromatographic method. The alkali hydrolysis product of dichlorvos reacts with the reagent to form pink colour compound.



Chandegaonkar*et al*.[53], has developed another spray reagent for detection of monocrotophos from biological material. A sensitive and selective thin layer chromatographic method for the detection and identification of monocrotophos using benzyl is described. The alkali hydrolysis product of monocrotophos reacts with benzyl to produce a pink colour compound.

Prabhavale S.S. *et al*.[54] have reported a thin layer chromatographic detection of organo- phosphorous insecticide containing nitrophenyl group. These insecticides on reduction with stannous chloride in 50 % hydrochloric acid give respective amino derivatives. These amino derivatives further react with iminodibenzyl yield purple coloured complex.

U.D. Pawar *et al.*[69] had reported thin layer chromatographic detection of Profenophos by cupric ferrocyanide reagent.

Lanjewar R.B. *et al* [55] developeda new chromogenic reagent for the detection of Profenophos by HPTLC. In this paper reagent consisting of sodium hydroxide, potassium ferricyanide and o-Toluidine used for detection of Profenophos.

Kulkarni *et al.*[56] has studied Indoxacarb, an oxadiazine insecticide, is described by High performance thin layer chromatographic method. Indoxacarb on acid hydrolysis yield its oxadiazine derivative**.** The oxadiazine under acidic condition react with diacetylmonoxime and furnish azide derivative of oxadiazine which is being sensitive to heat and light turns black on heating.

Chandegaonkar*et al*.[57]has reporteda sensitive and selective thin layer chromatographic method for the detection and identification of dichlorvos using mercuric (II) chloride is described. The alkali hydrolysis product of dichlorvos reacts with mercuric (II) chloride to produce a black colour spot.

Kulkarni *et al.*[58] has reported a thin layer chromatographic method for the detection of Hydrogen cyanamidein alkaline medium followed by the reagent of sodium nitroprusside and potassium ferricyanide. Chandegaonkar*et al*.[57] has studied Thin-Layer Chromatographic Detection and Identification of the Insecticide Imidacloprid. *P*- dimethylaminobenzaldehyde was found to be suitable for detection and identification of imidacloprid in routine forensic toxicological analysis.

Chandegaonkar*et al*.[59] has reported a thin layer chromatographic method for the detection of cypermethrin using p-benzoquinone in DMSO (Dimethyl sulfoxide). The alkali hydrolysis product of cypermethrin reacts with p-benzoquinone reagent to form a blue colored spot.

Some other pesticides such as Hydrogen Cyanamide,Indoxacarb, Imidacloprid also reported by TLC in biological material.

High-performance liquid chromatography (HPLC) has also been used to measure levels of imidacloprid residues in water and soil (Baskaran *et al*. 1997).[60]Baskaran *et al*. (1997) suggest that levels of imidacloprid cannot be determined directly using gas chromatography as a result of its thermo labile and polar *N*-nitroguanidinyl moiety. Volatility may also be increased as a result of the substitution of the acidic hydrogen of the NH at the 3-position of the imidazolidine ring (Baskaran *et al*. 1997).

The analytical methods that are currently being used for detection and measurement of imidacloprid were differing in their applicability to different types of environmental media, and also in the detection levels that can be achieved.

Concentrations of imidacloprid in water and soil can be measured using a GC-MS technique (Vilchez et al. 1996).Samples of imidacloprid are transformed into a volatile compound through hydrolysis in a basic medium. Using a liquid-liquid extraction with chloroform will allow for sufficient extraction and pre-concentration of the hydrolysis product. The detection limits using this technique have been reported as 0.16 µg/L for water and 1 µg/kg for soil (Vilchez *et al*.1996).

Detection of pesticides in non-biological material by TLC[61,62]GLC[63,64] HPLC[63]has been also reported in the literature. A large number of gas-liquid chromatographic methods[64] for residue analysis of synthetic pyrethroid have been reported.[61] These methods applied for residue analysis of pyrethroid in grain, water sample, fruit, vegetables, soil and insecticidal formulation where interfering sub stances are very less.

Kulkarni *et al*[66].has reported unusual finding of cypermethrin in flour.

Jivraj Makadiya1 *et al*.[67]has reported monitory study in which milk samples collected from different animals were analysed for the adulteration by different chemicals and also for the presence of pesticides residues. The purpose of this study was to check whether milk was contaminated with

pesticides or not. This study will be helpful for general public and farmers that they should use pesticides with caution.

E. H. Elgailani *et al.*[68] reported the levels of pesticide residues of acephate 75% SP in some vegetable samples in Albaha local area, Saudi Arabia.

Pesticides may occur in foods in concentrations called trace levels. Trace levels are generally at concentrations of parts per million, that is, one microgram of pesticide per gram of food or less. Measuring such small amounts of pesticides in the presence of enormous amounts of other chemicals that occur naturally in food is a challenge because those chemicals may interfere with measurement, A variety of analytical methods (see Ch. 6) are currently used to detect pesticide residues

It is also observed from the above literature survey that a very few chromogenic reagents are reported for specific and selective detection of pesticides and drugs of forensic interest by TLC & HPTLC. Among the reported reagent, most of these are described for the detection and determination of these compounds in non-biological materials like grains, vegetables, fruits, water samples, soils etc. shows the interference with co-extracted biological materials like fats, proteins peptides and amino acids etc.

The traditionally used Dragandroffs like chromogenic reagent are widely used for detection of basic nitrogen containing compounds. [47] However, it is observed that there are many limitations for this reagent. Therefore, there is a need to develop different chromogenic reagents and analytical method for detection of newly invented pesticides, drugs and other organic compounds of forensic interest.

Hence an attempt is being made to develop a new chromogenic reagent and analytical methods for selective detection of pesticides and drugs of forensic interest from biological and non-biological materials.

Thus, there is a need to developvarious types of chromogenic reagents

for specific and selective detection of drugs, insecticide and other organic compounds of forensic interest by TLC & HPTLC, having less/no interference with co-extractives.

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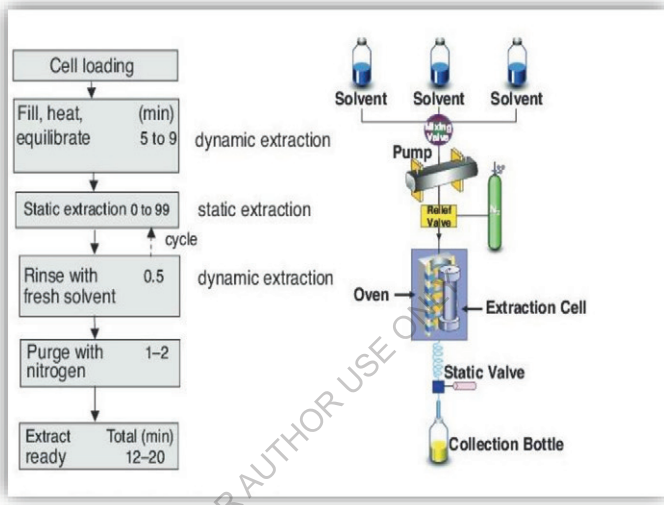
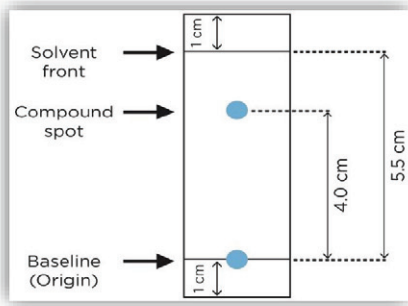
Chapter II

Development of method for isolation and extraction of drugs and pesticides from biological materials

Section A





Section A:

2. Introduction:

Toxicology is an interdisciplinary field dealing with adverse (toxic) effects of exogenous chemical substances (xenobiotics) on living organism. Toxicology covers two aspects: **medical**, which focuses on the effects of poisons and optimal therapy of intoxications, and **analytical,** which deals with demonstration and determination of amount of usually unknown substance or mixture of substances and their metabolites in biological or non-biological material.

As poisons (toxic, poisonous substances) are denoted substances that following penetration to the organism are able to produce an injury even in a relatively small amount. Also, drugs can act as poisons if overdosed. Toxicologically significant substances include a wide array of substances with diverse physico-chemical properties.[1,11]

- Drugs, pesticides, insecticides, herbicides, addictive substances;
- Volatilesubstances (e.g. ethanol, methanol, toluene, trichlorethylene);
- gases (e.g. carbon monoxide, hydrogen cyanide);
- inorganic acids and bases;
- metals (e.g. arsenic, lead, mercury).

2.1 Biological material:

In toxicological analysis a diverse material is processed. It is important to send the material in sufficient amount so that in case of poisoning with an unknown toxin as many analyses as possible can be performed. The usually examined kinds of materials are the following.[3,6,7,8:]

• **Stomach content** – at least 50 ml of vomits or the first portion of gastric lavage. Analysis of gastric content is useful in an early stage of

intoxication when a yet unabsorbed substance can be present. In stomach content it is possible to find the drugs in their original forms.

• **Blood, or serum (10 ml**) – level in blood is affected by the time passed since entry of the noxa (= harmful substance) to the body, rate of its absorption and excretion. Concentrations of poisons in blood are usually low. Blood is suitable especially for quantitative analysis

• **Urine** – poison appears in urine later than in blood, but also persists longer, and so analysis of urine gains significance in later stages of intoxications. The noxious substance is often concentrated in urine, but it can be present as a range of its metabolites rather than in the original form. That is why analysis of urine can be more demanding. It is the most suitable material for capture and identification of poison. At least 100 ml is needed for analysis.

• **Biological material obtained after death** – contents of the gastrointestinal tract, tissue samples, and body fluids.

• **Material secured in relation to intoxication (non-biological materials**)– includes pills, liquids, injection syringes, and food remnants.

The isolation of toxic compounds from material of biological origin is very complicated and multistage process. There are three stages of poisonous substance isolation from objects of chemical-toxicological investigation. On each stage there are many factors, which influence on isolation efficiency.[1,4,5]

I stage. Infusing of investigated objects with isolating liquids: – character and method of object preparation, – nature of isolating liquid and applied electrolyte (acid or base), – pH of solution, – recurrence and duration of infusing, – mode of peptides precipitation, – mode of impurities separation.

II stage. Extraction with organic solvents: –

-nature of organic solvent, –

- pKa of substance, – For acid substances pH = pKa – 2

- pH of solution, -- For base substances pH = pKa + 101

-distribution coefficient,

– nature and concentration of electrolyte, – ionic strength of solution.

III stage. Concentration and purification (clarification) of isolated substance: – chosen purification technique,

– re-extraction, – chromatography,

– solid phase extraction,

– sublimation.

2.1.1Classical method for extraction of non-volatile organic poison:

Solvent Extraction, Stas-Otto, Digestion with ammonium sulphate, sodium tungstate or other modified methods.

2.1.2 Modern method for extraction of non-volatile organic poison:

Paired ion extraction chromatography, HPTLC, supercritical fluid chromatography, solid phase extraction, micellar extraction, affinity chromatography, microwave assisted reaction system, accelerated solvent extraction, sweep co-distillation universal trace residue extraction.

The success of any screening procedure is directly related to the efficacy of the extraction procedure for the compound(s) of interest. The ideal general screening procedures require that the extraction process extract all the compounds of interest present. The use of solvent extraction procedures typically targets compounds of similar polarity and solubility, and multiple extractions with various solvents would have to be performed to cover all the compounds of interest.

2.2 Solvent extraction:

FIGURE 13:SCHEMATIC DRAWING OF A SEPARATORY FUNNEL WITH TWO DISTINCT LAYERS OF LIQUIDS

Solvent extraction is a technique extensively utilized in both industrial applications and in the laboratory. It includes a variety of techniques such as liquid extraction (LLE), liquid solid extraction (LSE), supercritical fluid extraction (SFE), and other special techniques. LLE is an extraction technique applied to liquids, liquid samples, or samples in solution, using a liquid extracting medium. In sample preparation for chromatography, the technique is used for separation purposes.

The procedure is typically done using a separatory funnel (see Figure 1). After a short period of shaking (frequently using mechanical shakers), the two layers of liquid are allowed to separate. The layer of interest is then physically separated (taken aside) and, if necessary, the extraction is repeated on the remaining liquid.

2.2.1 Liquid-liquid Extraction (LLE): -

Liquid-liquid extraction of drugs and other lipophilic poisons from the specimen into an appropriate, water-immiscible, organic solvent, usually at a controlled pH, is widely used in analytical toxicology. Solvent extraction removes water and dissolved interfering compounds, and reduction in volume (by evaporation) of the extract before analysis provides a simple means of concentrating the compounds of interest and thus enhancing sensitivity.

Some form of mechanical mixing of the aqueous and organic phases is normally necessary. Of the methods available, vortex-mixing is the quickest and the most efficient for relatively small volumes. Rotary mixers capable of accepting tubes of up to 30 ml in volume are valuable for performing relatively large volume extracts of plasma/ serum, urine, or stomach contents, and serve to minimize the risk of emulsion formation. Centrifugation in a bench-top centrifuge, again capable of accepting test-tubes of up to 30 ml in volume and attaining speeds of 2000-3000 rev/min,

is normally effective in separating the phases so that the organic extract can be removed. Ideally, the centrifuge should have a sealed motor unit (which is flash proof) and tubes should be sealed to minimize both the risk of explosion from ignition of solvent vapour and the risks associated with centrifugation of infective specimens. Finally, filtration of the organic extract through silicone-treated phase-separating paper prevents contamination of the extract with small amounts of aqueous phase.

2.2.2 Solid-phase extraction (SPE): -

SPE is an extraction method that uses a solid phase and a liquid phase to isolate one, or one type, of analyte from a solution. It is usually used to clean up a sample before using a chromatographic or other analytical method to quantify the amount of analyte(s) in the sample. The general procedure is to load a solution onto the SPE phase, wash away undesired components, and then washes (elutes) off the desired analytes with another solvent into a collection tube. The goal of SPE is to quantitatively remove an analyte from a sample matrix with complete recovery in a solvent so that the recovered analyte is suitable for subsequent analysis. Owing to the limitations of most commercially available SPE sorbents, this ideal is rarely achieved.

2.3 Stas-Otto method:

As there are too many steps in the extraction of non-volatile organic poison by Stas-Otto method and also chances of loss of poison in each of the steps, there may be even a complete failure to detect it in case of considerable loss of poisons. The objectives of modifications are to minimize the steps as far as practicable under special circumstances. The following modifications of the technique are sometimes necessary.

i. The alcoholic extraction of biological materials is to be carried out at room temperature (not exceeding 40 0C) i.e. without using steam bath and preferably with absolute alcohol (to prevent hydrolysis) in place of rectified spirit for suspected poisoning by aconite, belladonna, Datura or cocaine. The evaporation of alcoholic extract should be done under reduced pressure

ii. The extraction with rectified spirit is to be done for 48 hours if biological materials are preserved in saturated saline solution.

iii. For stomach contents comprise off too much of fluid, the extraction should be done with absolute alcohol for 3 or 4 times.

iv. For stomach wash as the sample, extraction with double the quantity of absolute alcohol acidulated by tartaric acid should be done and then allowed to evaporate on a steam bath.

v. For filtration, Buchner funnel is preferred.

vi. To prevent loss due to emulsion formation, agitation with organic solvents, (ether or chloroform or amyl alcohol) should be done gently in the beginning and steadily in a violent manner thereafter (2–3 time in the beginning and not exceeding 12 times at the end). If emulsion persists, it is to be evaporated on a steam bath and the residue taken up in fresh solvent.

2.4 Accelerated Solvent Extractor:

Accelerated Solvent Extraction (ASE) [13] is an extraction method that significantly streamlines sample preparation. A commonly used solvent is pumped into an extraction cell containing the sample, which is then brought to an elevated temperature and pressure. Minutes later, the extract is transferred from the heated cell to a standard collection vial for clean-up or analysis. The entire extraction process is fully automated and performed in minutes for fast and easy extraction with low solvent consumption. Up to 24 samples can be loaded and extracted sequentially without requiring operator intervention. ASE is used in Forensic Science laboratory for extraction of pesticides and drugs in viscera, sediment, soil, dry waste etc.

2.4.1 Extraction of Pesticides in Sediment, Soil, Dry Waste and Tissue by Accelerated Solvent Extraction (ASE):

The optimum analytical condition for extraction of pesticides in biological material has been developed using Accelerated Solvent Extraction (ASE) based on the principal of sweep co-distillation

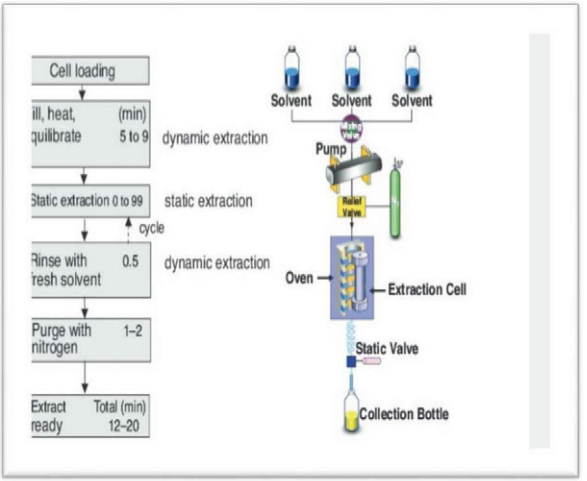


FIGURE 14:ACCELERATED SOLVENT EXTRACTOR

2.4.2 Procedure:

Extraction of pesticides in biological material by accelerated solvent extraction (methods as per technical notes of M/S Dionex). The biological sample is mixed thoroughly or passed through a 1 mm sieve. Sufficient sample is introduced into the grinding apparatus to yield at least 10-20 gm after grinding. The sample is air dried at room temperature for 48 hours in a glass tray or on hexane cleaned aluminium foil. The drying may also be made by mixing with anhydrous sodium sulphate until a free-flowing powder is obtained (air drying is not recommended for volatile pesticides). Gummy, fibrous or oily materials not amenable to grinding should be cut, shredded or otherwise separated to allow mixing. These may be grinded after mixing with anhydrous sodium sulphate 1:1 proportion. A cellulose disk is placed at the outlet and end of the extraction cell. Approximately 10 gm of each sample in 11 ml or 20 gm of each sample in 22 ml extraction cell (surrogate spikes and matrix spikes may be added to the appropriate sample cell). The extraction cells were placed into the auto-sample tray and the collection trays are loaded in appropriate number (up to 24). The tray is loaded with 40 ml pre-cleaned, clapped vials with septa. The conditions for extraction in ASE are set for extraction of pesticides by

using acetone: hexane (1:1 v/v) as the solvent. The operating conditions include oven temp of 100 0C, pressure at 1500 psi, oven heat time and static time each of 5 minutes and flush volume in the proportion of 60% of extraction cell volume. (Fig 1) The extracts are collected for analysis. The method has been validated for analysis of pesticides in soil, sediment, dry wastes and fish tissues. However, further standardization is required for application of ASE to biological matrices in forensic toxicological work.

2.5 Extraction of Pesticides:

The procedures for extraction, isolation and clean up for analysis of pesticides in different biological material have been described below. Solvent extraction procedure is the most frequent method of choice which is simple and easily available; namely, as a concentration step, as a clean-up procedure and to render the sample in a form suitable for analysis.

2.5.1 Method I: Extraction of Pesticides in stomach-wash, urine and vomit:

The sample (20 ml of stomach wash or urine or10- 20 gm of vomit) is taken in a conical flask and 50 ml of n-hexane is added. It is refluxed on a water bath for half an-hour. After cooling, the liquid is filtered, mixed with 20 ml of n-hexane and taken in a separating funnel. The n-hexane layer is separated; passed through anhydrous sodium sulphate and evaporated to dryness by passing a current of dry air through it.

2.5.2 Method II: Extraction of Pesticides in Blood:

20 ml of blood is mixed with 10 ml of 10% sodium tungstate solution and 15 ml of 1N sulphuric acid, shaken for two minutes and then filtered. The filtrate is kept reserved. The residue is washed with two 15 ml portions of 0.1N sulphuric acid. The washings are collected, mixed with filtrate (kept reserved), transferred into a separating funnel and extracted thrice with 20 ml portion of n-hexane. The hexane layers are combined and passed through anhydrous sodium sulphate and the solvent is removed by passing a stream of air as stated in the previous methods.

2.5.3 Method III: Extraction of Drugs in Urine:

Sufficient phosphoric acid or tartaric acid is added to 10 ml of urine to adjust the PH to 3. It extracted with two 30 ml of ether. The extract is combined and washed with 5 ml of water. The washings are added to the sample. The aqueous solution is retained for possible presence -salicylates (Fraction A)

The ethereal solution of extracted with 5 ml of 0.5 M sodium hydroxide and the extract is retained for examination of barbiturates, paracetamol (Fraction B).

The ethereal solution is washed with water. The washing is discarded. The ethereal solution is then dried over anhydrous sodium sulphate and evaporated to dryness. The residue may contain neutral drugs Lorazepam, Flurazepam, Nitrazepam, paracetamol (Fraction C). The aqueous solution retained after the first extraction sufficient dilute

2.5.4 Extraction of Drugs in Blood:

As sample volume in case of blood or serum or plasma is small and only limited number of drugs may easily be detected and identified in them, the extraction procedure is slightly different from that for urine and stomach contents. Different fractions of extraction viz. A, B, C, D, bear the same meaning as stated above i.e. extraction in urine. The initial extraction is carried out pH 7.4 as drugs are recovered by chloroform extraction at this pH. As the result substances looked for is most likely to be found in either fraction B or C and preparation of fraction D is only necessary either to ensure that nothing has been missed or where no drug has been found in fraction B and C.

Procedure: 2 ml of phosphate buffer (pH=7.4) and 40 ml of chloroform are added to 4 ml of the sample and shaken vigorously. 2 gms of anhydrous sodium sulphate is added and shaken again to produce a solid cake. The decant chloroform is passed through a filter paper and the cake is extracted with a further 20 ml of chloroform. The chloroform extract is combined.

The chloroform layer is extracted with sodium carbonate to remove salicylate ((Strong Acid Fraction A), if detected in the preliminary tests. To

the chloroform layer, 8 ml. of 0.5 N sodium hydroxide solution is added. The mixture is shaken and centrifuged. The sodium hydroxide may contain barbiturates and other weakly acid substances. (Weak acid fraction B)

The chloroform layer is washed with a little water. The washing is discarded. The chloroform layer is dried over anhydrous sodium sulphate, filtered and evaporated to dryness. The residue may contain (caffeine, carbromal, benzodiazepines, meprobamate, phenazone, etc.) neutral drugs together with a number of bases (Neutral and Basic Fraction C).

If sufficient of original sample is available, a further portion of it is made alkaline with dilute ammonia solution and extracted with two 10 ml portion of chloroform. The chloroform extract is dried over anhydrous sodium sulphate and evaporated to dryness. The residue may contain basic drugs (Basic fraction D- as stated above).

If there is not sufficient of the original sample for the extraction of basic fraction F, the following procedure may be carried out. After fraction C has been chemically examined by UV or chromatographic methods, the remaining residue, if any dissolved in chloroform and extracted with 0.5 M sulphuric acid. This extracted portion is added to the sodium sulphate cake retained after the first extraction (For fraction A) It is made alkaline with dilute ammonia solution and extracted with two 10 ml portions of chloroforms. The chloroforms layer is collected, dried over anhydrous sodium sulphate and evaporated to dryness, the residue may contain basic drugs (Basic fraction D.)

2.6. Analytical methods used in Forensic toxicology for the detection of drugs and forensic interest compounds:

Deaths due to poisoning, to be proven beyond all reasonable doubt for the purpose of the law, requires the combined effort of three experts, namely, the Investigating police officer, the autopsy surgeon, and the chemical examiner. The report of the chemical examiner naturally becomes the most important evidence as it indicates the presence of poison both in qualitative and quantitative way

2.6. (A): Methods of detection of poisonous substances, isolated from biological material:

Table 1:

Chemical	
Colour tests	Volatile poisons, alkaloids, medicines, metal poisons, pesticides, acids, bases
Precipitation reactions	
Microcrystalline reactions	
Physicochemical	
Thin layer chromatography	Alkaloids, medicines, pesticides
Gas chromatography	
High performance liquid chromatography	
UV-VIS Spectroscopy	Alkaloids, medicines
Mass-spectroscopy	

2.6.(B): Methods ofquantitative determinationof poisonous substances isolated from biological material:

Table 2:

Chemical	
Gravimetric Titrimetric	Volatile poisons, metal poisons, pesticides, mineral acids, salts, bases
Physicochemical	
Photometry	Volatile poisons, alkaloids, medicines, pesticides, metal poisons, salts
Direct and differential spectroscopy	Alkaloids, medicines
Extraction-photometry	Alkaloids, medicines, metal poisons, pesticides
Gas-liquid chromatography	Volatile poisons, pesticides
Biochemical	
Enzymatic	Volatile poisons, pesticides

Methods of detection of poisonous (drugs, pesticides) substances, isolated from biological and non-biological materials are—

2.6.1. Colour Test:

A colour test is a chemical procedure in which the substance tested is acted on by a reagent, which causes a change in the reagent, thereby producing a characteristic colour or colour change. The greatest

utility of colour tests in toxicology is the rapid screening of urine specimens, as the urine may be analysed directly without time-consuming extraction procedures. A false positive result, that is the development of a colour when the correct compound is not present, is one of the drawbacks of this method. The forensic analyst must be aware of the limitations of this method and particularly the sources of false-positive reactions.[14]

2.6.2. Chromatography:

Chromatography is the most modern versatile method used for separation and purification of organic compounds. The method was first discovered by Tswett, Russian botanist, in 1906 for the separation of colour substances into individual's components. In chromatography separation is achieved by the differential movement of individual components through a stationery phase under the influence of mobile phase.

The components of a sample mixture are distributed between two phases, one of which is stationary while the second one, the mobile phase, percolates through a matrix or over the surface of a fixed phase. Although there are many varieties of chromatographic analysis the three most commonly applied by toxicologists and forensic analysts are thin-layer chromatography (TLC), gas chromatography (GC) and high-performance liquid chromatography (HPLC).[14]

2.6.3. Thin Layer Chromatography:

TLC involves separation of components of a mixture over a thin layer of an adsorbent. A thin layer of an adsorbent is spread over a glass plate of suitable size, the plate is called as TLC plate. The TLC has found wide recognition in many fields and its sensitivity of detection offers particular advantage to the toxicologist which has increased ten to hundred times as compared to the chemical methods. [2]

In TLC, the stationary phase is a "thin layer" of an absorbent, usually silica gel, which is spread on a solid support. Concentrated sample extracts and drug standards are applied as a series of spots along the bottom of the plate and placed in a closed tank in which the absorbent layer makes contact with the "developing solvent" (mobile phase) below the applied

spots. The solvent moves up the plate by capillary action, dissolving and separating the components of the extracts. The presence of drug is visualised by spraying or dipping into the various reagents, which produce coloured reactions with particular compound.

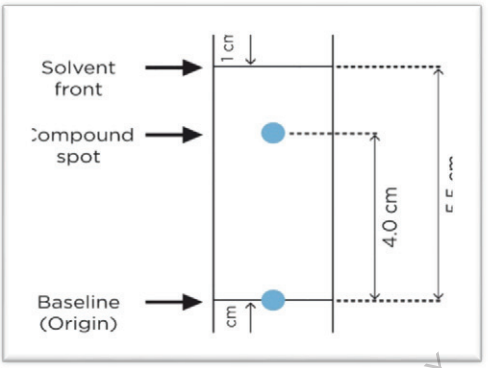


FIGURE 15:THIN LAYER CHROMATOGRAPHY

There are numerous TLC spray reagents to choose from, but the toxicologist must be guided by the chemical nature of the compounds of interest. If a compound from the extract migrates the same distance and reacts to the applied sprays in the same manner as the reference drug / compound, the toxicologist then has a tentative identification of the compound, which must be confirmed by another chemical or analytical test.

2.6.4. Gas chromatography:

Gaschromatography is basically a separation technique, in which the compounds of a vaporized sample are separated and fractionated as a consequence of partition between mobile gases phase and a stationary phase held in the column. Partition takes place between gas and liquid or gas or solid. Thus, according to the nature of stationary phase, GC may be divided into two classes: Gas Solid chromatography, Gas Liquid chromatography.[2]

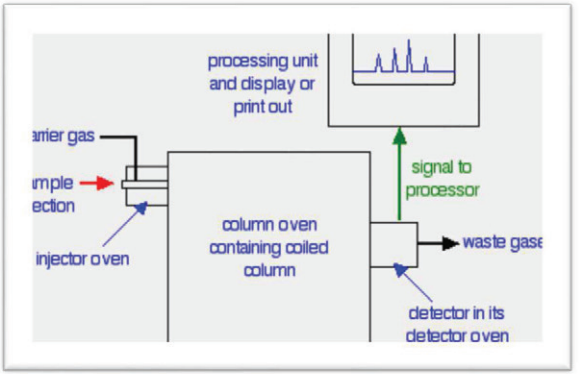


FIGURE 16:SCHEMATIC DIAGRAM OF GAS LIQUID CHROMATOGRAPHY

GLC is preferably used for analysis of volatile and thermo-stable pesticides in recent years. Capillary columns have almost completely replaced the packed columns owing to their high resolving power, which allows the separation of large number of [pesticides with similar characteristics' most frequently used detector includes, ECD, NPD, FPD and MSD.

Quantitative works usually requires some form of sample preparation to isolate the drug from the bulk of the material for interference free analysis. For the purpose, some degree of concentration or dilution is required. These steps or process will certainly invite some degree of analytical error. A wide range of basic drugs including alkaloids may be screened or detected by Gaschromatography.

2.6.5 High Performance Liquid Chromatography:

HPLC is a unique separation technique for separation of organic components of mixture, developed by Kirkland and Huber in 1969.It is defined as the separation technique involving mass transfer between stationary and mobile phase.

The modern form of column chromatography has been called high performance chromatography, high-pressure, high-resolution and high-speed liquid chromatography. However, the abbreviation HPLC is now universally understood to describe the technique that separates mixtures on columns filled with small particles (typically 10 μm or less in diameter) by elution with a liquid under pressure. The essential equipment consists of an

eluent reservoir, a high-pressure pump, an injector for introducing the sample, a stainless-steel column containing the packing material, a detector and a data recording device. Thus, HPLC is similar to other types of chromatography in having a stationary phase (packing material) and a mobile phase (eluent) [12] HPLC is being extensively used now a day in analysis of drugs and forensic investigations due to its high specificity and sensitivity. HPLC can detect large number of thermally labile molecules which can't be analysed by GLC.HPLC is applied in detection and separation of substances on the basis of their chemical nature.

HPLC plays a significant role in analysing the pesticides contents in the blood, tissues and drinks. There has also been occupational disease due to chronic exposure to pesticides but contaminated ground water, In the news, there were also allegations of detecting pesticides in soft drinks, like coca cola.

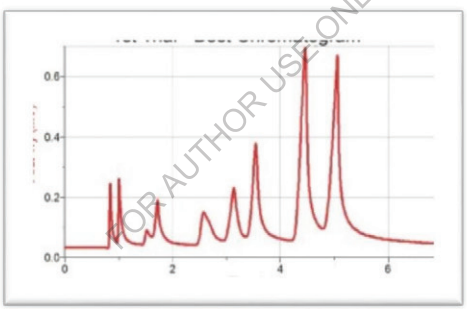


FIGURE 17:TYPICAL CHROMATOGRAM m

HPLC methods are used in screening, identification and quantification of several drugs of forensic samples. These drugs may be basic, acidic or neutral. Heroin brown, LSD green, amphetamine blue or yellow, tetrazine, cannabis etc these and many other chemicals are analysed by HPLC techniques in forensic interest compounds However, today HPLC has proved to be a valuable. techniques for forensic analysis. It is effective in almost all organic compounds such as pesticides, herbicides, insecticides, acidic drugs, basic drugs, snake venom, plant poison etc.

2.6.6. Workflow of toxicological analysis:

The workflow of toxicological analysis includes several stages (fig.6):

Screening – based on immunochemical methods, thin-layer chromatography together with the system of colour reactions (CR), gas and liquid chromatography[11].

Demonstrations (identification) of a chemical entity – more sophisticated methods are usually needed. The analysis employs liquid and gas chromatography with mass detection, thin-layer chromatography with various developing systems, sorbents and detection agents, and other methods.

Quantitative estimation of exogenous substance in biological material.

Evaluation of findings by a toxicologist and physician.

In the further explanation we will focus mostly on the first stage of toxicological analysis

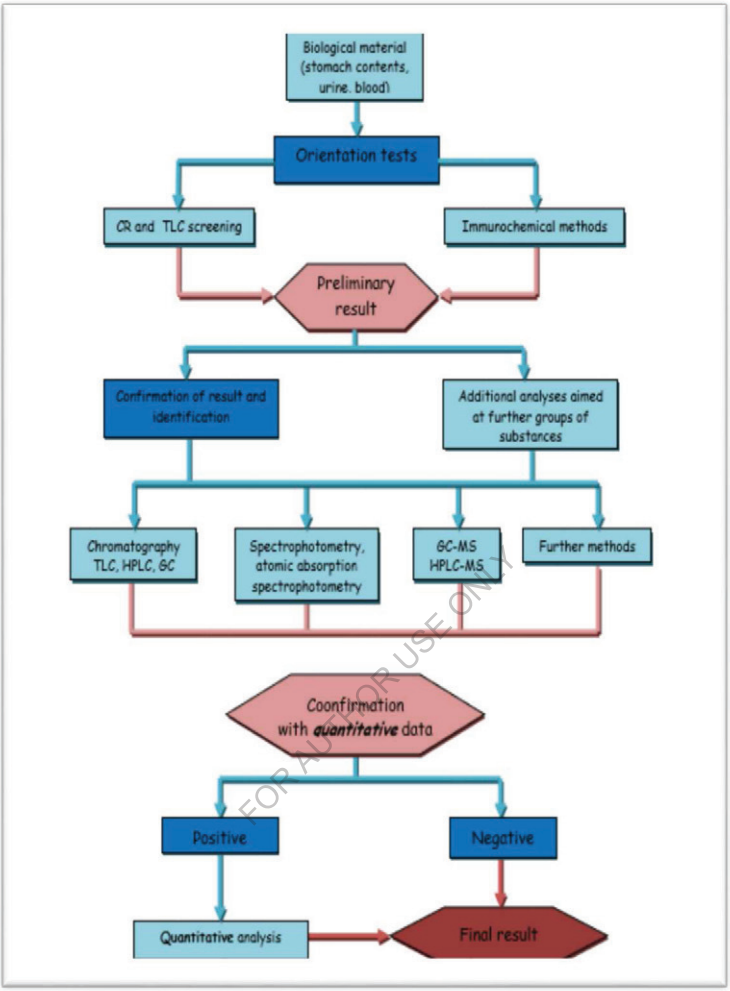


FIGURE 18:SCHEME OF THE WORKFLOW IN TOXICOLOGICAL ANALYSIS

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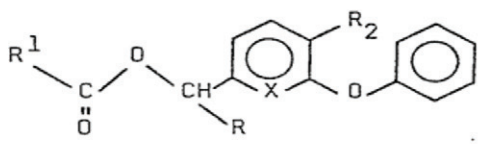
A New Chromogenic Reagent for Carbamate Insecticides

Section B



Journal of Planar Chromatography 29 (2016) 6

A New Chromogenic Reagent for Carbamate Insecticides

Ulka K. Kulkarni*, Krishna V. Kulkarni, Rajendra K. Pardeshi, and Dhananjay V. Mane

Key Words:

Insecticides
Toxicological
High-performance thin-layer chromatography

Introduction

Carbamates (carbaryl, baygon, carbofuran; **Figures 1 and 2**) belong to a family of chemicals that kill or control the insect known as carbamate. These insecticides are widely used against a broad spectrum of insects on field crops, fruits, and vegetables and against household flies and mosquitoes [1]. New varieties of these insecticides are easily available. Due to their easy availability, insecticides are often misused in homicidal and suicidal cases, requiring toxicological examination.

Figure 1

The chemical structure of carbamate.

Section B

2.7 Introduction:

Carbamate insecticides are a group of compounds closely related to Organophosphate insecticides in chemical structure, mode of action, and many other properties. These compounds inhibit the enzyme acetylcholine esterase (AChE) in the neuromuscular junction and cause the death of the organism by neuromuscular paralysis.

Physostigmine is a naturally occurring inhibitor of AChE, known to western medicine at least since the 19th century. It was isolated from the Calabar bean, *Physostigma venenosum*, in 1864, and its structure was known by 1923. Physostigmine was the first drug against glaucoma and myasthenia gravis; although it has mostly been superseded by synthetic derivatives, physostigmine is still used to diagnose myasthenia.

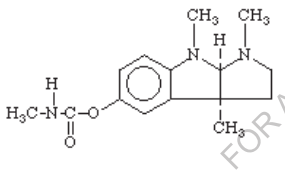


FIGURE 20: PHYSOSTIGMINESTRUCT

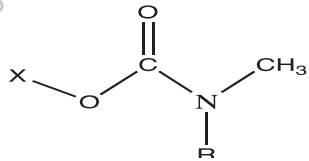


FIGURE21: GENERALIZED CARBAMATE

The quaternary nitrogen compounds, including physostigmine and acetylcholine itself, have no insecticidal activity, apparently because they cannot penetrate the lipid sheath of the insect nerve (due to the formally charged quaternary nitrogen). RL Metcalf, R Fukuto, and their colleagues at Riverside began with the structure of physostigmine, and tested related *uncharged* structures (notably the isomeric dimethyl aminophenol *N*-methyl carbamates) to find potential insecticides. Theirstructure-activity experiments also used information about the structure of AChE that indicated that, in insects, approximately 5 A separate the anionic from the esteratic site.

This work was subsequently used by Bayer AG to develop the first commercial carbamate insecticides. Thus, the carbamate insecticides were the first class of insecticides derived from deliberate considerations of structure-activity relationshipsInvestigations of chemicals that exert an anticholinesterase action on the nervous system similar to organophosphates led in the 1950s to the development of the carbamate insecticides. Carbamate insecticides are derivatives of carbamic acid, HOC(O)NH2. They have the general formula shown below where R is an alcohol, oxime or phenol and R1 is hydrogen or a methyl group.

The general formula of the carbamates is:

Figure 3. General Formula of carbamates

where R^1 and R^2 are alkyl or aryl groups.

Carbamates are: carbaryl, carbofuran, Propoxur, Aldicarb, Methomyl.

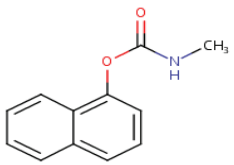


Figure 4: Chemical structure of carbaryl

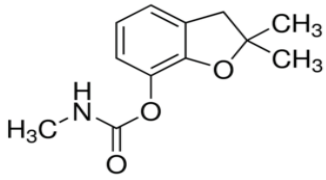


FIGURE5: CHEMICALSTRUCTUREOFCARBOFURAN

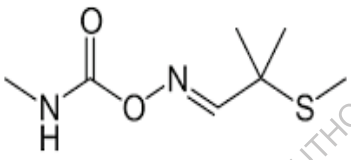


Figure 6: Chemical structure of Aldicarb

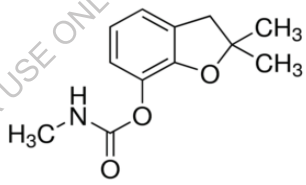


FIGURE 5: CHEMICAL STRUCTURE OF CARBOFURAN

Carbamates (carbaryl, propoxur, carbofuran; **Fig. 4,5,6 and 7**) belong to a family of chemicals that kill or control the insect known as carbamate. These insecticides are widely used against a broad spectrum of insects on field crops, fruits, and vegetables and against household flies and mosquitoes. New varieties of these insecticides are easily available. Due to their easy availability, insecticides are often misused in homicidal and suicidal cases, requiring toxicological examination

Forensic toxicologists need to be able to characterize these insecticides. A number of analytical and advanced instrumental methods, i.e., gas chromatography, gas chromatography and mass spectroscopy, as well as high-performance liquid chromatography have been reported for the easy detection of carbamates from biological and non-biological materials.

Though these techniques are rapid, specific, and sensitive, they cannot be always used for the detection of insecticides which are extracted from biological materials, as the purity of samples is in question.

Hence, in routine forensic toxicological examination, high-performance thin-layer chromatography (HPTLC) is the best technique for the identification and detection of insecticides from biological materials.

2. 7.1 Literature Survey:

A number of reagents have been widely used for the detection and identification of carbamate insecticides, viz., diazo phenol (after alkaline hydrolysis) [1], alkaline fast blue-B [2], and Tollens' reagent [3]. The use of alkaline phenyl hydrazine hydrochloride [4], ammonium cerium nitrate [5], copper chloride (III), ammonium metavanadate [6], diazotized p-amino-1-naphthol-3-sulfonic acid, sodium hydroxide followed by a mixture of sodium bromide and copper chloride [7], and 4-aminoantipyrine followed by potassium ferricyanide [8] is reported to be specific for carbaryl only. diazotized-p-amino-1-naphthol-3-sulfonic acid (J. acid) and then spraying with NaOH [9] followed by observation under 366 nm UV light[10] are reported to be specific for carbaryl only. 0.5% diazotized p-nitroaniline in 1:4 HCL and then NaOH solution[10] and p-nitro benzene diazonium tetra fluoroborate reagent.[12,13] Alkaline hydrolysis followed by potassium ferricyanide. [14] Phloroglucinol (tautomeric compound) in acidic medium [15] are reported to be specific for methomyl. Alkyl hydrolysis followed by Folin Ciacaltau (FC).[16]

However, they are not very sensitive; the spots are ill-defined for low concentration of insecticides, and they cannot be easily located, possibly because of biological impurities.

2.7.2 Present Work:

A number of analytical and advanced instrumental methods, i.e., gas chromatography, gas chromatography and mass spectroscopy, as well as high-performance liquid chromatography have been reported for the easy detection of carbamates from biological and non-biological materials. Though these techniques are rapid, specific, and sensitive, they cannot be

always used for the detection of insecticides which are extracted from biological materials, as the purity of samples is in question. Hence, in routine forensic toxicological examination, high-performance thin-layer chromatography (HPTLC) is the best technique for the identification and detection of insecticides from biological materials.

In the present study, the authors have made efforts to use a new chromogenic spray reagent for the high-performance thin-layer chromatographic detection of carbamates. In this work, we used 10% aq. sodium hydroxide followed by 5% of toluidine reagent and 10% of aq. sodium nitrate, giving orange and violet spots.

2.7.3Chemicals and Reagents:

All the chemicals were of analytical grade. Distilled water was used throughout the analysis.

(a) 10% aq. sodium hydroxide: 10% (v/v) sodium hydroxide was prepared by dissolving 10 g of sodium hydroxide pellets in 100 mL of distilled water.

(b) o-Toluidine reagent: 5 g of o-toluidine in 14 mL HCl was filled up to 100 mL distilled water.

(c) 10% aq. sodium nitrate: 10% aqueous sodium nitrate solution was prepared freshly.

Standard solutions of carbaryl (1 mg mL−1), carbofuran (1 mg mL−1), and baygon (1 mg mL−1) were prepared in ethanol. Similarly, the entire standards (Profenophos, thiodan, and cypermethrin) were also prepared in ethanol.

2.7.4 Extraction Procedure:

An automated system for extracting organic compounds from a variety of solid and semisolid samples was used. If the sample contains water, then diatomaceous earth is added to absorb the water contents and get a solid and semisolid sample for extraction. ASE 200 accelerates the traditional extraction process by using solvent at elevated temperature. Pressure is applied to the sample extraction cell to maintain the heated solvent in a liquid state during the extraction. After heating, the extract is flushed into the collection vials and prepared for analysis. Approximately 20 g of visceral sample such as stomach, intestine, liver, spleen, and kidney,

having a history of consumption of carbofuran insecticides, is cut into fine pieces along with diatomaceous earth and transferred into the extraction cell. The extracts were collected in a clean collection vial; diethyl ether was used for extraction at 50°C and 1000 psi pressure in two cycles. The extracts obtained were transferred into a steel capsule and evaporated to dryness at room temperature. The residue was dissolved in 2 mL of ethanol and processed further by HPTLC.

2.7.5 Experimental section:

2.7.5.1 High-Performance Thin-Layer Chromatography:

Chromatography was performed on 10 cm × 10 cm silica gel 60 F254 HPTLC glass plate (Merck, Darmstadt, Germany). A CAMAG (Muttenz, Switzerland) Linomat IV applicator was used to apply 10 mL in ethanol equivalent to 10 mg along with standard carbaryl, carbofuran, baygon, and extract of viscera having a history of death due to the consumption of carbofuran. Blank viscera, Profenophos (organophosphorus), thiodan (organochlorine), and cypermethrin (pyrethroid) were applied on HPTLC plate. The plate was then developed in presaturated 24 cm × 8 cm × 22.5 cm CAMAG twin-trough TLC chamber to a distance of 10 cm using hexane–ethyl acetate (9:1 v/v) as the mobile phase. The plate was removed from the chamber, dried in air, and sprayed with 10% sodium hydroxide solution followed by o-toluidine reagent combined with 10% sodium nitrate by using a glass sprayer. Successively, orange and blue-violet spots were observed at RF values shown in Table 3.

Table 3:

No	Name of Insecticides	Colour	Rf.value
1	Baygon	Orange Faint violet	0.48 0.51
2	Carbaryl	Orange	0.46
3	Carbofuran	Violet Violet	0.46 0.49
4	Viscera having a history of death due to the consumption of carbofuran	Violet	0.49

2.7.6 Recovery Experiments:

Carbaryl, baygon, and carbofuran (each 1 mg in ethanol) were separately added to the minced visceral tissue (50 g) mixed well and left to stand for 24 h. The tissue samples were then processed as above (Extraction Procedure) except that the residue from the extraction of the tissue was dissolved in 1 mL of ethanol. This solution (10 mL) was spotted on a separate activated plate with the respective standard solution of 10 mL of carbaryl containing 7, 8, 9, 9.5, and 10 mg in 10 mL ethanol. The plates were then developed and processed as described above. The intensity of the spot obtained for the extracts of the visceral tissue was compared with that from the standard and found to be most similar to the spot resulting from the 8 mg (10 mL) −1 standard solution of carbaryl. Hence, the recovery for carbaryl was 80%.

2.7.7 Results and Discussion:

In this reaction (Figure 3), after alkaline hydrolysis of carbofuran, 1-napthol is formed. This is coupled with diazotized o-toluidine to form azo dye.

This reagent is selective for carbamates. Other organophosphorus insecticides such as Profenophos, organochlorines such as thiodan, and pyrethroids such as cypermethrin do not give colour spot. Moreover, the constituents of viscera (amino acids, peptides, and proteins) which were generally coextracted with the insecticide do not interfere. The sensitivity of the reagent is ca. 0.2 mg per spot as observed after development

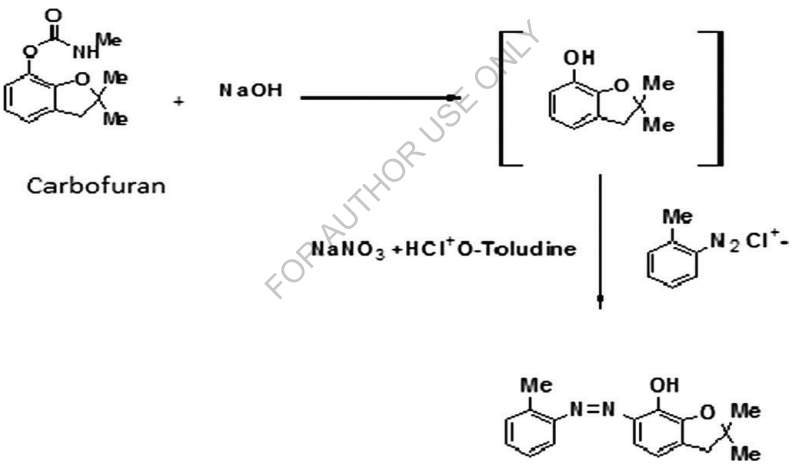


FIGURE 8: PROBABLE CHEMICAL REACTION OF CARBAMATE

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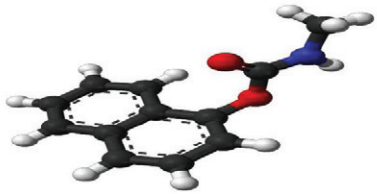
A Specific Spray reagent for the identification and detection of carbaryl in biological materials

Section C



Chemical Structure of Carbaryl

JPC **Short Communications**

A Specific Spray Reagent for the Identification and Detection of Carbaryl in Biological Materials

Ulka K. Kulkarni, Krishna V. Kulkarni, Rajendra K. Pardeshi, and Dhananjay V. Mane*

Key Words:

Insecticide
High-performance thin-layer chromatography

1 Introduction

Carbaryl (1-naphthyl-N-methylcarbamate; Figure 1) belongs to a family of chemicals that kill or control insects, known as carbamates. These insecticides are widely used against a broad spectrum of insects on field crops, fruits, and vegetables and against household flies and mosquitoes [1]. A number of reagents have been used for their detection and identification, viz., diazophenol (after alkaline hydrolysis), alkaline fast blue-B [2], Tollens' reagent [3] has been widely used for the detection of carbamate insecticides. The use of alkaline phenylhydrazine hydrochloride [4], ammonium cerium nitrate [5], copper chloride(III), ammonium metavanadate [6], diazotized 6-amino-1-napthol-3-sulfonic acid (J-acid) [7], and NaOH is reported to be specific for carbaryl only. However, it is not very sensitive, the spots are ill-defined for low concentration of insecticides, and they cannot be easily located, possibly because of biological impurities.

It is therefore necessary to have a sensitive reag
baryl. In this paper, we report the use of 10% N
a mixture of sodium bromide and copper chlor
formance thin-layer chromatography (HPTL
identification of carbaryl insecticide with a sol
ane and ethyl acetate (9:1).

2 Experimental

2.1 Chemicals and Reagents

All the chemicals were of analytical grade. Di
used throughout the analysis. Ten-percent (v/v)
ide was prepared by dissolving 10 g of sodium
in 100 mL of distilled water.

(a) An amount of 5 g of sodium bromide was di
of distilled water. (b) An amount of 5 g of cop

Section C

2.8 Introduction:

Carbaryl is a carbamate pesticide that was first registered for use in the United States on cotton in 1959, the company now in famous for the Bhopal disaster of 1984, carbaryl is synthesized through treating methyl isocyanate with 1-napthol. The resulting product is a solid, usually colourless crystal that is odourless and soluble in water.[1]

Carbaryl (chemical name 1-naphthalenyl methylcarbamate, CAS No. 63-25-2) is sold under many trade names, the most common being Sevin, and other names are Propoxur (Baygon) Carbofuran (Furadan). Today, carbaryl is a widely used broad-spectrum insecticide used in agriculture, professional turf management, ornamental production, and residential settings and against household flies and mosquitoes. The primary mechanism of action is reversible inhibition of acetylcholinesterase and it is generally regarded as being safe with respect to human health.[2]

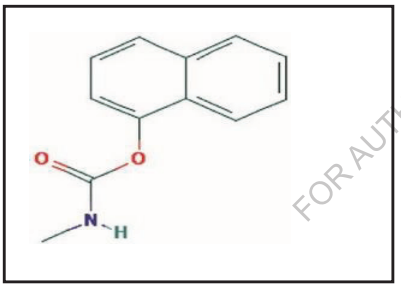


FIGURE1; THE CHEMICAL STRUCTURE OF CARBARYL

Over the years, a large dossier of toxicity, environmental fate, residue, and monitoring data have been generated, which, coupled with practical use experience, have been used to improve application methods and to refine exposure and risk assessments that support the continued registration and safe use of this insecticide.

Carbaryl is a dangerous chemical, being an anticholinesterase compound, a suspected carcinogen, teratogen and a skin irritant, and it is also dangerous to fish and wild life. It may pose special problem for people

eating a low protein diet. [3,4] Although carbaryl is acknowledged to be moderately hazardous, even by its proponents, one of the intermediators products, methyl isocyanate, is known to be instantly toxic and regarded as very dangerous.

However, because intermediate substances do not require the same safetyclearances as formulated pesticides, neither Union Carbide, nor United Nations agencies monitoring toxic substances, knew very much about its toxicity before the accident at Union Carbide, Bhopal (chemical plant mainly producing carbaryl). [5]

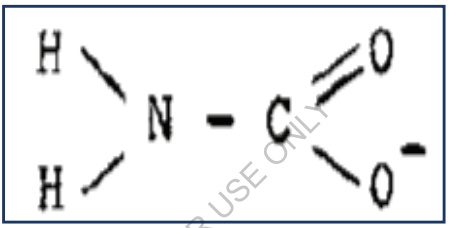


FIGURE 2: CHEMICAL STRUCTURE OF CARBARYL

The toxicity of carbaryl arises from the combination of atoms known as the carbamate group, Compounds containing the amino (-NH2) and carboxy (-COOH) groups are termed amino acids. Carbamic acid is an example of an amino acid and carbaryl is the derivative of carbamic acid. At least 26 different amino acids are known to be essential to the structure of protein. [6] An upset of the balance of these structures in the body by carbamic acid derivatives cause sickness and death.

The carbamate pesticides namely carbaryl, carbofuran, methomyl, propoxur, thiophanate methyl and cartap are widely used for the same purposes. Different manufacturing companies prepare this carbamate group of pesticides due to low manufacturing cost and highly effective nature of them and so these carbamates are available in the market.

The wide usage of carbaryl in agriculture for protection of crops is continually increasing and this is reflecting in the increasing number of criminal cases referred to forensic science laboratories concerning to be total 510 misuse of this compound for homicidal, suicidal and sometime in accidental poisoning. Identification of these pesticides becomes essential to protect the ecology/mankind from the adverse effect of pesticide residues and forensic toxicology and criminology purposes.[7]

Number of analytical and advanced instrumental methods viz. UV, gas chromatography and mass spectroscopy, HPLC can be used for the identification of these compounds. Though these techniques are rapid, specific and sensitive, but these techniques cannot be used for identification of compound extracted from biological matrix as the purity of sample is in question. Hence for routine toxicological examination TLC and spot test are more practical technique for the identification of sample extracted from biological and non-biological matrix like soil, food, plant and human tissues. A several numbers of solvent system and chromogenic reagents are available for the detection of pesticides. The identification of carbamate group of pesticides namely carbaryl (1-napthyl-N-methyl carbamate), carbofuran (2,3,-dihydro-2,2-dimethyl-benzofuran-7-dimethylcarbamates),methomyl(o-methyl-thioacetohydroxamate),propoxur(2-isopropoxy-N-methycarbamate), thiophanate methyl(dimethyl-4,4'-(o-phenylene)-bis-(3-thioalophanate) and cartap[s,s'-(2, 2'-dimethyl-amino trimethylene-bis-thio-carbamate)] by using TLC techniques for routine work are reported. [8-9]

2.8.1 Literature Survey:

A number of reagents have been used for their detection and identification, viz., diazo phenol (after alkaline hydrolysis), alkaline fast blue-B [10]; Tollens's reagent [11] has been widely used for the detection of carbamate insecticides. The use of alkaline phenyl hydrazine hydrochloride [12], ammonium cerium nitrate [13], copper chloride (III), ammonium metavanadate [14], diazotized 6-amino- 1-napthol-3-sulfonic acid (J-acid) [15], and NaOH is reported to be specific for carbaryl only. However, it is not

very sensitive; the spots are ill-defined for low concentration of insecticides, and they cannot be easily located, possibly because of biological impurities.

2.8.2Present Work:

It is therefore necessary to have a sensitive reagent to detect carbaryl in biological materials. In this paper, we report the use of 10% NaOH solution followed by a mixture of sodium bromide and copper chloride for high-performance thin-layer chromatographic (HPTLC) detection and identification of carbaryl insecticide with a solvent system hexane and ethyl acetate (9:1).

2.8.3 Chemicals and Reagents:

All the chemicals were of analytical grade. Distilled water was used throughout the analysis.

1) **Preparation of sample solution**: A standard solution of 1mg/ml strength of commonly used carbamate insecticides namely carbaryl, carbofuran, carbosulphan were separately prepared in methanol,

2) **Preparation of reagents:**

Ten-percent (*v*/*v*) sodium hydroxide was prepared by dissolving 10 g of sodium hydroxide pellets in 100 mL of distilled water.

(a) Sodium Bromide Solution: An amount of 5 g of sodium bromide was dissolved in 50 mL of distilled water.

(b) Copper Chloride Solution: An amount of 5 g of copper chloride was dissolved in 50 mL of distilled water.

2.8.4 Extraction Procedure:

Extraction of carbaryl from biological material:

An automated system for extracting organic compounds from a variety of solid and semisolid samples was used. If the sample contains water, then diatomaceous earth is added to absorb the water contents and get a solid and semisolid sample for extraction. ASE 200 accelerates the traditional extraction process by using solvent at elevated temperature. Pressure is applied to the sample extraction cell to maintain the heated solvent in a liquid state during the extraction. After heating, the extract is

flushed into the collection vials and prepared for analysis.[16] Approximately an amount of 20 g of visceral sample such as stomach, intestine, liver, spleen, and kidney, having a history of consumption of carbaryl insecticides, was cut into fine pieces along with diatomaceous earth and transferred into the extraction cell. The extract was collected in a clean collection vial; diethyl ether was used for extraction at 50°C and 1000 psi pressure in two cycles. The extract obtained was transferred into a steel capsule and evaporated to dryness at room temperature. The residue was dissolved in 2 mL of ethanol and processed further by HPTLC.

2.8.5 Experimental section:

2.8.5.1 High-Performance Thin-Layer Chromatography:

Chromatography was performed on 10 cm × 10 cm silica gel 60 F_{254} HPTLC glass plate (Merck, Darmstadt, Germany). A CAMAG (Muttenz, Switzerland) Linomat IV applicator was used to apply 10 µL in ethanol equivalent to 10 µg along with carbaryl extract of viscera having a history of death due to the consumption of carbaryl, blank viscera, std. Carbofuran, std. Carbosulfan **(Figure3)**andon another plate Profenophos (organophosphate). Thiodan (organochlorine) and cypermethrin (pyrethroid) were also applied on HPTLC plate along with standard carbaryl. The plate was then developed in presaturated (24 cm × 8 cm × 22.5 cm) CAMAG twin-through TLC chamber to a distance of 10 cm using ethyl acetate–hexane (9:1, *v*/*v*) as the mobile phase. The plate was removed from the chamber, dried in air, and sprayed with 10% NaOH solution followed by a mixture of (a) and (b) solution by using a glass sprayer. Successively blue-violet spots were observed at R_f value 0.51 for std. carbaryl and viscera having a history of death due to carbaryl. Other carbamates such as carbofuran and baygon did not give colour spot.

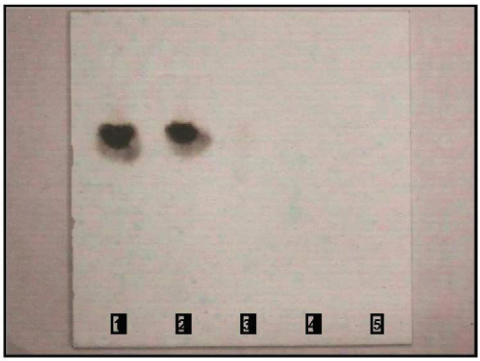


FIGURE 3: CHROMATOGRAM OBTAINED FROM CARBARYL INSECTICIDES

1. Carbaryl from extract of viscera Standard Carbaryl
2. Standard Carbaryl
3. Blank viscera
4. Standard Carbofuran
5. Standard Carbosulfan

Where R= aryl group

2.8.6 Recovery Experiments:

Carbaryl (1 mg in ethanol) was separately added to the minced visceral tissue (50 g), mixed well, and left to stand for 24 h. The tissue samples were then processed as above (Extraction Procedure) except that the residue from extraction of the tissue was dissolved in 1 mL of ethanol. This solution (10 µL) was spotted on separate activated plate with the respective standard solution of 10µL of carbaryl containing 7, 8, 9, 9.5, and 10 mg in 10 mL ethanol. The plates were then developed and processed as described above. The intensity of the spot obtained for the extracts of the visceral tissue was compared with that from the standard and found to be

most similar to the spot resulting from the 8 mg (10 mL) $^{-1}$ std. solution of carbaryl. Hence, the recovery for carbaryl was 80%.

2.8.7 Semi-quantitative determination of Carbaryl:

Carbarylwas semi quantitatively determined in biological and non-biological materials by HPTLC with visual assessment. Carbaryl was extracted by using chloroform: alcohol (7:3) from known amount of (50 gm) of biological sample such as viscera, blood, stomach-wash etc. and non-biological materials such as grains, food materials, water sample, soil etc. as described under 'extraction of Carbaryl".[17]The extract was then evaporated at room temperature and the residue was dissolved in 1-2 ml ethanol. A 10 µl volume of this extract was spotted on HPTLC plate together with 10 µl each of standard solution of technical carbaryl containing known concentration of 1, 5, 10, 15, 20, 25…. mg per 10 ml in ethanol. The plate was then developed as described under "chromatography procedure" and sprayed with 10% sodium hydroxide solution followed by mixture of sodium bromide solution and copper chloride solution.. The intensity of blue-violet colored spot was developed for the extract of unknown concentration, was visually compared with those of known standards. From this the amount of carbaryl present in the total extract and that in the 100 gm of viscera was determined. Since visual assessment was done, it is a semi-quantitative determination.

2.8.8 Results and Discussion:

This reagent is selective for carbaryl. Other carbamates such as carbofuran and baygon, organophosphorus insecticides such as Profenophos, organochlorines such as thiodan, and pyrethroids such as cypermethrin do not give colour spot. Moreover, constituents of viscera (amino acids, peptides, and proteins) which are generally coextracted with the insecticide do not interfere. The sensitivity of the reagent is ca. 0.5 µg per spot observed after development.

On alkaline hydrolysis, carbaryl yields 1-naphthol which reacts with copper chloride and sodium bromide to give a violet bluish complex. The colour of spot is stable for a couple of days. The reagent described here

is very sensitive and specific for carbaryl, and hence, it can be used routinely for the detection and identification of carbaryl and its breakdown product 1-naphthol in biological and non-biological material in forensic toxicology.

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Chapter III

Detection of Poisonous Plants from autopsy Tissues

Section A

3. Introduction:

In the context of biology, poisons are substances that can cause disturbances to organisms. Throughout human history, intentional application of poison has been used as a method of assassination, murder, suicide, and execution. Poison includes both naturally produced compounds and chemicals manufactured by humans. Natural poisons are produced by species of bacteria, fungi, protists**, plants** and animals. Poisonous plants are those which cause serious problems or even death occur, if a small quantity of its stem, leaves, seeds, fruits and roots are ingested [3]. Some other plants are normally harmless but they may become toxic if preparative from

them are taken in excess in strong doses or for a long period of time as suggested by Qureshi et al. [4].

Ayurvedic, Siddha and Unani have been in existence in India from ancient times. Siddha system is capable of treating all types of diseases by using poisonous plants. These plants comprise the third largest category of poisons known around world. According to World Health Organization (WHO), approximately 80% of the population of developing countries depends on plant drugs, plant medicines for regular source of medicines, either in part or entirely. For many this is out of necessity because they cannot afford the high cost of pharmaceutical drugs or do not have access to university-trained medical practitioners

Any substances can be harmful only at high concentration- as Paracelsus (1493-1541) said, the dose make the poison. In India studies on poisonous plants has been done by Chopra et al (1949,1956,1984), Islam (1986, 1996), Desai (1999), Caisus (1986), Kumar and Sikarwar (2003) et al. Poisonous principles are classified based on the chemistry of toxic compounds present in it: Alkaloids, Glycosides, Oxalates, Photosensitizing compounds, Phytotoxins, Polypeptides and Resins. Plants differ by degree of toxicity and classify them as extremely, moderately or minimally toxic. It is difficult to categorize plants with regard to their toxicity, since this varies with the age of the victim, environment, and stage of plant growth. Degree of toxicity is variable within a plant or plant family.

There are more than 4000 species of medicinal plants growing as herbs, shrubs, and trees in India, many of which are poisonous when administered in large doses. The toxic principles belong to alkaloids, glycosides, toxalbumins, resind, cannabinoids and polypeptides. Suicide in India, as poison can be easily obtained and many poisonous plants grow wild, e.g. datura, oleanders, aconite, nux vomica, etc. Many Indians consider the taking of life by blood-shed a greater crime than poisoning, strangling etc. Accidental poisoning occurs from the use of philters or love potions and quack remedies containing poisonous drugs. The incidence of poisoning in India is among the highest in the world, and it is estimated that

more than 50,000 people die every year from toxic exposure [3]. The causes of poisoning are many – civilian and industrial, accidental and deliberate. One recent study pertaining to poisoning statistics demonstrated more of such differences between northern and southern Indian states [11].

In India, mostly in rural areas, mostly plant poisons are used for robbery and suicidal purposes. For example, Datura is used by that sect of the thugs who poisoned wayfarers. Even today the poisoning and robbing of travellers was of frequent occurrence in India. By the judicious use of datura a whole household can be so drugged that the thieves can ransack the house at their ease. Datura has frequently been detected in the vomit of the victims of a midnight robbery. Red chilli powder is frequently used in the robbery or a confession of some guilt by introducing it into the nostrils, eyes, urethra, vagina, or rectum. Hyocyamus is used in war to control shell-shock. Poisoning is generally accidental though an overdose or rarely homicidal as in the Crippen case.Hemlock was Athenian state-poison by which Socrates died.

It is essential to take cognizance of the fact that overuse or abuse of the medicinal constituents of plants can cause danger [9]. Plants containing glucosides, acids or alkaloids are used as medicines. Thus, when taken in excess often have adverse effect. The latex, white or coloured sap found in families of Apocynaceae, Asclepiadaceae, Sapotaceae, Euphorbiaceae and Papaveraceae, if used in excess always act as poison. Plants of family Araneae have calcium carbonate oxalate crystals, which cause intense irritation of mouth and throat, as also swelling of throat and intestinal lining. This may cause suffocation or death. Some plants containing orthophosphoric acids cause painful irritation and eruption if they came in contact with skin or mucous membrane. There are some plants or products like seeds of Annona squamosa L. and unripe pineapple when consumed induce abortion in pregnant women

Hence poisoning due to toxic plan is a great problem in rural area. Every year, Forensic science laboratory receives some cases of plant poisoning. There is a challenge to words forensic expert to detect the plant

toxin in autopsy tissue. The review on all toxic plants has been summarized in the present study which provide a fundamental database for the forensic community which has been reviewed with the available literature [12 – 18], those who are working the field of forensic crime scene and as well as toxicologist as standard comparison during laboratory examination, which plants contains which type of constituents in it Even plant toxins were quite low in incidence, which is because of the difficulty in testing for such toxins in the laboratory, as compared to chemicals.

This study combined, for the first time, forensic investigation, chemistry and botany to create a unique platform needed for the identification of poisonous plants and their components in forensic exhibits, blood, urine, stomach wash and viscera. The research was focused on the poisonous plants previously detected at the laboratory, as well as the requests received for the analysis of multi/toxic plant components. The selection of plants included Nicotiana glauca, Datura stramonium / Datura ferox, Callilepis laureola, Boophone disticha / Ammo Charis coranica, Abrus precatorius, Ricinus communis, Nerium oleander / Thevetin peruviana and Bowiea volubilis. All these species are known to have caused fatalities, hence their choice.

Chapter III

Some Reagents for Detection and Identification of Poisonous Plant in Autopsy Tissues

Section A

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Some Reagents For Detection And Identification Of Poisonous Plant In Autopsy Tissues

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Abstract: This paper deals with the use of high Performance Thin layer Chromatography with sensitive spray reagents for detection and identification of poisonous plants in autopsy tissues. This laboratory has received human as well as cattle cases with history of death due to ingestion of poisonous plant material in various forms. There can be legal quarries regarding detection and identification of these poisonous plants, which a forensic expert has to answer. In the present paper, a very little effort has been taken to provide a perfect database for forensic toxicologist which can be useful for detection of poisonous plant.

Keywords: High performance thin layer chromatography, autopsy tissues.

700 poisonous plant species belonging to encountered in India include over 90 botanical families Common poisonous plants encountered in India includes—

- ✓ Irritants plant: castor, glory lily, Croton, marking nut, may apple, red pepper, rosary pea.
- ✓ Cardio toxic plants: Aconites, Autumn crocus, Common oleander, yellow oleander, Suicide tree.
- ✓ Neotoxic plant: Calotropis, Cassava datura, Shychos.
- ✓ Hepatotoxic Plants: Neem
- ✓ Miscellaneous toxic plants: Arecanut, marking nut

Plant poisoning may be accidental poisoning, it also acts in criminal offences in Indian penal code (IPC). Accidental poisoning with some of these plants or plant products may occurs among inhabitants of rural areas, dependent on their forms and gardens for food, due to mistake in identifying toxic

Section A

3.1 Introduction:

A poison is a substance which when administered, inhaled or ingested is capable of acting deleteriously on the human body. Thus, there are really no limits, between medicine and a poison. For medicine, in a toxic dose is a poison and a poison, in a small dose may be a medicine. Means, it depends on dose/quantity only. The incidence of poisoning is among the highest in the world, and it is estimated that more than 50,000people die every year from toxic exposure. The cause of poisoning is many-civilian and industrial, accidental and deliberate. The commonest agents in India appears to be pesticides, insecticides (organophosphorous, Carbamate, Organo chloro, Pyrethroids) chemical compounds, corrosive acids, drugs, alcohols and plant toxins. In India there are over 400 poisonous plant species belonging to encountered include over 90 botanical families. Common poisonous plants encountered in India includes—

- Irritants plant: castor, glory lily, Croton, marking nut, may apple, red pepper, rosary pea.
- Cardio toxic plants: Aconites, Autumn crocus, Common oleander, yellow oleander, Suicide tree.
- Neotoxic plant: Calotropis, Cassava datura, Shychos.
- Hepatotoxic Plants: Neem
- Miscellaneous toxic plants: Arecanut, marking nut

Plant poisoning may be accidental poisoning, it also acts in criminal offences in Indian penal code (IPC). Accidental poisoning with some of these plants or plant products may occurs among inhabitants of rural areas, dependent on their forms and gardens for food, due to mistake in identifying toxic plants, with children being at particular risk. Contamination of foodstuff and the use of poisonous plants in traditional or folk medicine are other cause of poisoning. Suicide using poisonous plant is fairly common in India, especially in rural areas, most typically with cardiac glycosides containing fruits of yellow oleander or Suicide tree, both

of which are rarely employed in homicide. The administration of a poison is a criminal offence whenever 1) it is with intent to kill ii) used recklessly even though there is no intent to kill. iii)for stupefying to facilitate a crime i.e. Rape iv) to produce an illegal abortion v) to annoy the victim vi) with intent to cause serious injury.

Now a days, in India mostly, poisons are used for robbery purpose. Datura is used for this purpose. Even today the poisoning and rubbering of travellers was of frequent occurrence in India. By the judicial use of datura a whole household can be so dragged that the thieves can ransack the house as their ease. One case of datura poisoning was sent by Shirdi Police station. Datura seeds were mixed with Prasad. Datura has frequently been detected in the vomit of victim of midnight robbery. Marking nut is used as abortive agent in rural areas. Juice of marking is introduced into vagina in illegal abortion cases. Hyocyamus is used in war to control shell-shock. Hemlock was Athenian state –poison by which Socrates died.

In India, especially in Maharashtra state, besides above-mentioned cases, some cases of plant poisoning occur in cattle. Some of the plants are used for criminal purposes such as (vernacular names are parenthesis) oleander (white Kaner) Cerbra thevetin (pila kaner) and Calotropis, Gigantea (Madar)

Hence all the toxic plants which occurs in Maharashtra region, has been summarized in the table 1, This table provide a fundamental database for forensic toxicologist and the study provide valuable data for standard comparison. During laboratory examination, which plant contains which type of constituents in it. Poisons plants in India have been described by few people. A lot of work has been reported on identification and detection of poisonous plant but no work has been done in terms of forensic context. In this paper, we reported basic detail such as botanical and family name, toxic part of plant, chemical constituents, fatal dose and fatal period which is given in table 1. Effort has been taken to overcome method of detection of poison plants, spray reagents, mobile phase and sensitivity which is shown in table 4.

Table 4:

	Name of the plant/family	Common name	Toxic part	Toxic constituents	Fatal dose	Fatal period
1	Calotropis gigentea (Apocynaceae)	Calotropis and Madar	Juice and roots	Uscharin calotoxin, calactin and calotropin	0.12 mg/kg calotroping	12 to 24 hours
2	Cerbera thevetia (Apocyanac eae)	Yellow oleander and Pila Kaner	All parts specially leaves and fruits	Theretin, Thertoxin, Neri Folin peruvosid	8 to seeds, 5-10 leaves	Depending upon quantity
3	Conium maculatum (Apiacea)	Poisons hemlock	All parts	Conine and methyl conine	1 cm piece of plants	
4	Croton tiglium (Euphorbia)	Croton Oil seeds or Jamalgota	Seeds and oil	Crotin a toxal bumine Tigglic acid, crotoni c acid and crotonosids	4-6 seeds,1-2 ml. oil	
5	Datura fastuosa (Solanaceae)	Thom apple and Datura white oleander (Kaner)	All parts, especially seeds and fruits.	Atropine Hyoscyamine	0.6-1 gram	26 hours
6	Narium Odorum	White oleander (Kaner_	All parts	Hyseine and Dutarin, Neriodonge, Neriodorein and Keration	15-20 g. (24-36 h) 60-160 mg nicotine	5-15 min
7	Ochrocarpu s Longitolius (Gutileare)	Naag Kesar	All parts	Sorangin A and Sorangin B	Sorangium A 1 mg/kg, Sora nigum B-200 mg/kg	For cats
8	Pegnum harmala	Wild rise	All parts	Harmal Asicine Vasicinone	200 mg/kg	For Rabbit
9	Some carpus anacardium (Anacardiacea e)	Marking nut and bhilawa	Juice	Semecarlpo l and _Bhilawan ol	5-10 gm	12-24 hours
10	Strychhos nux-vomica (Loganiaceae	Poison nut and kuchila	All parts especially seeds	Strychnine brucine and romicin	15-20 mg/kg	1-2 hours
11	Cannabis Sativa	Indian Hamp, Hashish	Bhang dried leaves and fruits, Ganja flower, Charas,	Cannabin, cannabinoid and cannabinol	10 g, /kg bhang,8 gm/kg Ganja, 2gm/kg Charas	5- hours

			resin of leaves and stems.			

3.1.1 Experimental section:

Chemicals and reagents:

All the chemicals and solvents used were of analytical grade (Merck).

Distilled water was used throughout

Hexane, Acetone, methanol, chloroform - AR grade.

- p-dimethyl amino benzaldehyde- 200 gm. P-amino benzaldehyde in 10 ml conc. sulphuric acid
- Anthrone (9, 10 dihydro-8 oxanthrene) 2 gm. of Anthrone in 100 ml conc. sulphuric acid
- Sodium hydroxide solution: 10 gm. sodium hydroxide in 100 gm. Distilled water
- Dragandroffs reagent
- Idoplatinate reagent:
- Fast blue:
- Conc. sulphuric acid:

Some toxic constituents were isolated from plant material by stass otto process followed by acid chloroform extraction and evaporated at room temperature (27-ANerium Oleander plant was identified and samples of leaves, flowers and twings (without leaves) were collected. All the samples were air-dried at room temperature and grinded to particle size of 2-3 mm. The powdered plant material was stored in glass vials protected from light and humidity.

3.1.2 Extraction Procedure: Extraction was carried out by routine procedure.

3.1.3 High-Performance Thin-Layer Chromatography:

Chromatography was performed on 20 cm X 20 cm silica gel 60 F_{254} HPTLC glass plate (Merck). A camag (Switzerland), linomat IV

applicator was used to apply 10 µl extracted solution of Narium Odorum (Kaner), Madar, Datura, Marking nut along with autopsy tissues.

The plate was then developed in presaturated 24 cm X 22 cm Camag twin trough HPTLC chamber to a distance of 10 cm using Hexane: Acetone (7:3) (v/v) as mobile phase the plate was removed from the chamber, dried in air and sprayed with given spray reagents. Results are shown in Table No.5

Table 5:

SrNo.	Name of the Plant	Spray reagent	RF Value	Colour spot
1	Opium	Fast blue		Orange to violet red
2	Datura	P-dimethyl aminobenzene aldehyde	0.57	Violet
3	Datura (atropine)	Dragandroffs	0.45	Brown
4	Datura(atropine)	Idoplatinate	0.55	Violet blue
5	Oleander (white Kaner)	P-dimethyl Anthrone	0.38,0.50, 0.61	Pink green, blue green, blue green (all glycosides)
6	Oleander (white kaner)	Anthrone	0.50,0.54, 0. 57	Bluegreen, pink, blue (Nerine, Neriodorein, Neriodorein)
7	Oleander (pila kaner)	5%ferric Sulphate in acetic acid followed by H_2SO_4	0.34	Blue, Blue-green (Nerine), (Neriodorein)
8	Oleander (pila Kaner)	Anthrone	0.43	Blue –green (Cerberin)
9	Madar	P-dimethyl aminobenzene aldehyde	0.34,0.77	Violet brown, grey (Gigantea, Colour)
10	Madar	Conc. sulfuric acid	0.45	Reddish (Grgantin)
11	Madar	Anthrone	0.34,0.51 ,0.77	Orange, Dark brown, Blue-green- Uscharin, Cal act, Grgantin
12	Marking Nut	Sodium Hydroxide	0.55	blue

3.1.4 Result and Discussion:

Every year, FSL receives so many cases of various poisoning. The scope of analysis covers all the pesticides, herbicides, fungicides, volatile gases (HCN and Co exposure), blood alcohol and plant poison. Most of this compound received on fair amount of attention as to method development and adaption of equipment for the optimal detection of the compound of forensic interest, but the detection of poisonous plants was badly neglected. The reasons for this were multiple. Standards were not commercially available, there was a lack of accurate information as to plant used, plant part used dosage, toxicity and of suitable equipment

This Paper has elevated the analysis of poisonous plants which are most commonly available and widely used as poison. The approach and method of detection, identification during this study can be further expanded to cover a wider range of plant species for each of main classes of compounds (alkaloids, glycosides)

In this way, plant related forensic analysis in Maharashtra can become more sophisticated with a higher success rate in terms of the number of cases solved.

In this paper, more than 10 poisonous plant species belonging to number of families are given. Few of them are reported having Rf. Due to unavailability of sample, some plants cannot identify. Work on different poisonous plants and its reagents is in progress. The author hopes to report soon when work is over.

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Chapter III

High Performance thin Layer Chromatographic detection of cannabis in Forensic Interest

Section B

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High Performance Thin Layer Chromatographic Detection of Cannabis in Forensic Interest

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ABSTRACT

Cannabis, also known as Marijuana, and other forms of cannabis plant (bhang, ganja, and Charas) are very frequently submitted to forensic laboratories under THC narcotic drug and psychotropic substances act 1985. In routine cases the identification of Cannabinoids in marihuana is achieved unequivocally by the 'three-parameter

Section B

3.2 Introduction:

Cannabis or Hashish or hemp is an annual plant, known by the name: *Cannabis sativa* (F. Cannabinaceae). Generally, two varieties are grown: the fibre type which is cultivated mainly for fibre production and the drug type which is cultivated to provide the narcotic drug known by the name: *Marijuana (Marihuana) or Hashish.*

It contains tetrahydrocannabinol (delta-9-THC) and cannabinol (CBN), Cannabidiol also isolated (CBD). CBN is not present in the fresh plant and it is a measure of aging: as the sample ages, THC content declines and

decomposes to CBN, which means that fresh plants contain all a Cannabinoids in their acidic form (THA-acid or THCA, CBD-acid or CBDA etc.).

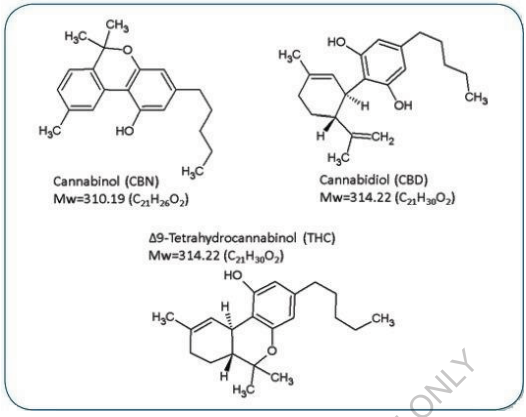

FIGURE 1: CHEMICAL CONSTITUENTS OF FORENSIC SIGNIFICANCE

Cannabis:

Chemical constituents of forensic significance

Cannabis and other forms of cannabis plant (bhang, Ganja, and Charas) are very frequently submitted to forensic laboratories under the narcotic drug and psychotropic substances act 1985.In routine cases the identification of Cannabinoids in marihuana is achieved unequivocally by the 'three-parameter approach' [Morphology, colour tests and thin-layer chromatography (TLC)] as suggested by Coutts and Jone[3]

The presence of cannabinoids is usually detected using colour tests[2], high performance liquid chromatography (HPLC) [3,4,5] gas chromatography (GC)[6]and commerciallyavailableimmunoassay based cassettes[7-11]. Following screening tests for cannabinoid detection, it is necessary to perform confirmatory tests using advanced techniques such as GC-MS, fluorescence polarization immunoassy[12], enzyme immunoassay[13] high performance thin layer chromatography (HPTLC)[14] and HPLC, which provide additional scope for quantitative monitoring of drugs during forensic analysis.

Although the instrumental methods are sensitive, they are expensive and there are limitations to their use in routine forensic work owing to the large number of samples (involving urine samples) to be handled. In this study we found that HPTLC method was found to be high - throughput, sensitive, reproducible and cost-effective compared to other methods

A number of chromogenic reagents such as Duquenois reagent[15],FastBlueSaltB[16],1nitrosonaphthol[17],FastBlueSalt2B[18]and2hydrazono2,dihydro3methylbenzothiazolehydrochloride (HMBT) [19, 20,] have been reported for the detection of Cannabinoids. Although Fast Blue Salt B as a chromogenic reagent seems to be the most commonly used reagent, its safety is questionable because of its potentialcarcinogenicity[21]. In a search for an alternative chromogenic reagent, P-Anisidine reagent in combination with ammonium metavanadate was found to be suitable for the detection of cannabinoids in marijuana.

3.2.1 Experimental Section:

Materials and Methods:

All the solvents used were of analytical-reagent grade. Distilled water was used throughout.

Sodium hydroxide: 10-gram sodium hydroxide pallets dissolved in 100 ml distilled water.

Spray reagent:

Solution: (a): saturated aqueous ammonium metavanadate

Solution: (b): Dissolve 0.5 g. p-Anisidine in 2ml. H_3PO_4, dilute up to 100 ml. with ethanol and filter.

For the extraction of cannabis, the cannabis sample (bhang, Ganja or Charas) was extracted with chloroform, the extract was filtered and evaporated to dryness and the residue was dissolved in chloroform for spotting. Reference standard (cannabinoids) was available in our laboratory.

3.2.2 High Performance Thin Layer Chromatography

Chromatography was performed on 10cm X 10cm silica gel60 F_{254} HPTLC glass plate [Merck], A camag [Switzerland], Linomat IV Applicator was used to apply 10 µl cannabis extract (Exhibits sent by police

authority under NDPS act) along with the standard reference solutions of cannabinol and cannabidiol pure reference standards dissolved in ethanol, on the plate, which was developed by the ascending technique in a presaturated chamber. After a run of about 10 cm, the plate was removed and allowed to dry at room temperature. It was sprayed uniformly with freshly prepared sodium hydroxide solution followed by solution (a). While plate is still wet, spray with solution (b). Immediately various colours appeared on plate. The R_F values of cannabinoids with respect to standards are given in Table I. Three solvent systems-(a) toluene: ethyl acetate: acetone (90:10:10), (b) ethyl acetate: methanol: ammonia (8.5:1:0.5), (c) petroleum ether: diethyl ether (4:1) were used.

3.2.3 UV spectra of THC, GCMS spectra of THC, CBD, CBN

The cannabis extract was spotted on a TLC plate, the plate was developed with either of the above solvent systems and one of the resolved spots was made visible by spraying ammonium metavanadate followed by p-anisidine solution. An equal area of silica gel layer was scraped off from a distance equal to the $R_F$value of A ‘-tetrahydrocannabinol (A ‘-THC), treated with 5 ml of ethanol and the solution mixed thoroughly. The solution was centrifuged and the UV spectrum of the supernant liquid was recorded. Same procedure has been done for GC-MS analysis for cannabinol, cannabidiol, and THC. (figure II)

3.2.4 Result and Discussion:

This study was done to develop a new chromogenic spray reagent for detection and separation of cannabis plant in forensic interest, this spray reagent can be routinely used to standardized protocol for detection of cannabinoids in the urine samples of person with cannabis abuse.

To further characterize the components of cannabis, the cannabis standard was subjected to HPTLC analysis in different solvent systems. Based on preliminary experiments, it was found that the cannabinoids exhibited different Rf values in different solvent systems (Table I). We selected THC, CBN and CBD as major cannabis constituents for detection of cannabis. HPTLC based separation of cannabinoids was done using

Toluene: ethyl acetate: acetone (9:1:1) solvent system. Fig. 1II A shows the chromatogram.

Most of the cannabinoids have a phenolic group with the ***ortho*** and ***para*** positions free. As phenols couple in the ***para*** position with diazonium salts, the cannabinoids also undergo similar reactions with ammonium metavanadate-p-anisidine reagent to yield coloured products. The cannabis extract gave approximately ten spots, a greenish yellow, yellow, violet spot corresponding to CBN, CBD and THC. (figure II)

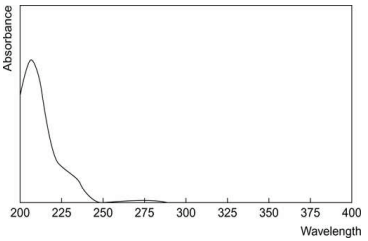

FIGURE 2: UV SPECTRA

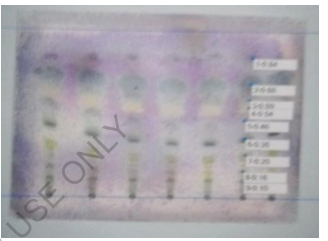

FIGURE 3: HPTLC PLATE

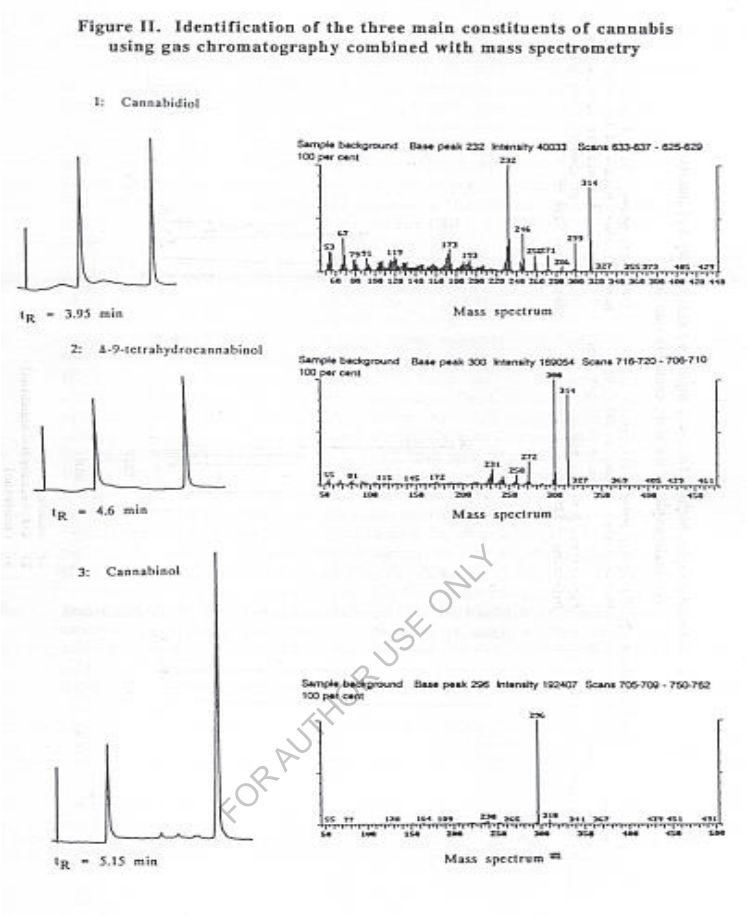

Table I

Rf values of cannabinoids in solvent systems: (a) Toluene: ethyl acetate: acetone (9:1:1) (b) ethyl acetate: methanol: ammonia (8.5:1:0.5) (c) petroleum ether: diethyl ether (4:1) plate sprayed with ammonium metavanadate –p-anisidine

Sr No.	Constituent	RF in a	RF in b	RF in c
1	CBN	0.59	0.27	0.28
	CBD	0.84	0.36	0.44
	THC	---	---	---

Although cannabis tests, based on commercially available ready-to use cassettes is available, it has limited utility. Because it is relatively expensive and has limited sensitivity threshold, and gives only qualitative and not absolute quantification. Most of the commercial kits clearly state that the test provides only a preliminary result and more specific alternative testing method should be used to confirm the immunoassay result[10]. This could be by either HPTLC or GC/MS or HPLC[6-10]. In routinely testing, on TLC RFvalue is not accurately recorded30. However, HPTLC plate gives good visualization. In this study, it was observed that the HPTLC based detection is best solution in forensic interest samples. It is a cost-effective, highly sensitive, and accurate up to the final data analysis and reporting only one hour is required). Further, with specific standards, and less time-consuming method. (following extraction, for 20 samples, starting from sample application) quantification is possible which could help to correlate the progress in rehabilitation/detoxification with the levels of cannabis in the urine samples.

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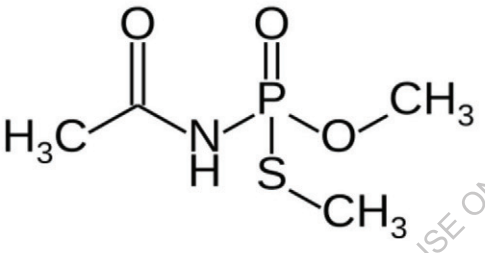
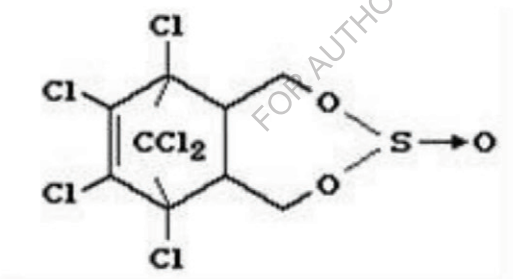
Chapter IV

Development of method for isolation and identification of newly invented pesticides





4 Introduction:

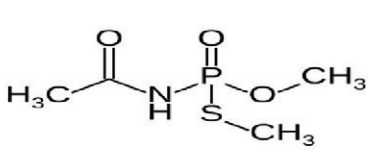
In present era, research and development work in agricultural pesticides is done in very large scale, because major investment of developing countries is in agricultural production and pest(insect) do not give response for older pesticides. Scientist develops new pesticides to yield more crops. Identification of these pesticides from biological material (viscera, blood, stomach wash) is bigger problem for forensic toxicologist. For this reason, we develop innovative methods for identification and detection of new pesticides

Pesticides, in general, are chemicals used worldwide in agricultural production to destroy or control weeds, insects, fungi, and other pests. Some of those pesticides remain on food as residues. When pesticides are applied improperly, resulting residues can pose significant health risks to consumers. Analysing for all pesticides on all types of food products is currently impossible because of limitations in testing methods as well as time and resourceconstraints.Althoughthenumberof pesticide/food combinations to address can be narrowed by focusing on the potentially moderate to high health hazard combinations, current analytical methods are not adequate to identify and quantify all residues of these pesticide/food combinations within available resources.

Chapter IV

New Chromogenic Spray Reagent for Detection of Acephate from Biological Material

Section A



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New Chromogenic Spray Reeagent for Detection of Acephate from Biological Material

U. K. Kulkarni, K. V. Kulkarni, D. V. Mane, R. K. Pardeshi

Abstract: High performance thin layer chromatography has found wide recognition in many fields and in sensitivity of detection offers particular advantage to the toxicologist, which has increased, 10 to 100 times as compared to the chemical method. It has become an important analytical tool since it can separate complex mixture in a relatively short time. In existing study, an effort has been taken to determine organ phosphorus insecticide Acephate by using high performance thin layer chromatography. A new specific Sensitive chromogenic reagent 0.1% solution of ferric chloride in 80% ethanol and 1 % Sulfosalicylic acid in 80% ethanol has been developed for detection of Acephate an organophosphorus insecticide with solvent system petroleum ether: methanol (95: 5).

Keyword: chromatography, chromogenic, Acephate, ferric chloride, ethanol, Sulfosalicylic, organophosphorus.

I. INTRODCTION

Generally Acephate (metamedophos) is widely used as organosphosphorus insecticide. The international union of pure and applied chemistry (IUPAC) chemical name for Acephate is O,S-Dimethyl acetylphosphoramidothioate. it is white crystalline transparence solid . has a strong odor This poisoning has claimed the largest number of victim in last fifteen years in Maharashtra state and hence in the forensic toxicology it has became necessary to identify organophosphorus insecticide as a group. In literature, a number of reagent such as mercuric nitrate followed by biphenyl carbazone (Joglekar1968)[3] mercuric nitrate followed by potassium ferrocynide (Katkar 1976)[4] Nesslers or tollens reagent (Kawale 1976)[5] alkaline resorcinol (Geiger,1976) NaOH and orthotoludine followed by potassium ferricynide (Lanjewar)[8] etc has been reported for the detection of organ phosphorus insect ides by thin layer chromatography. In existing paper we develop a new chromogenic spray reagent and new solvent system for the detection and identification of Acephate by HPTLC. The reagent consisting of 0.1% solution of ferric chloride in 80% ethanol and 1% Sulfosalicylic acid in 80% ethanol. Solvent system-(petroleum ether: methanol 95:5)

III. MATERIAL AND METHOD

Chemical and Reagent:

Section A

4.1 Introduction:

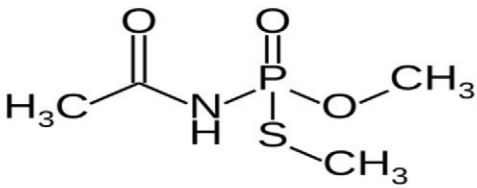

FIGURE 1: CHEMICAL STRUCTURE OF ACEPHATE

Acephate is an insecticide that belongs to the organophosphate group of chemicals. The international union of pure and applied chemistry (IUPAC) chemicalname for Acephate is O, S-Dimethyl acetyl phosphoramidothioate. it is white crystalline transparence solid, has a strong odour similar to mercaptan, which smells like sulphur.[1,2] Molecular weight- 186-16 g/mol. Solubility (water) -79-83.5 g/100ml. It is typically used as a foliar spray against chewing and sucking insects, such as aphids, leaf miners, Lepidopterous larvae, sawflies, and thrips on fruits, vegetables, potatoes, sugar beet, vines, rice, hops, ornamentals, and greenhouse crops like peppers and cucumbers. It can also be applied on food crops and citrus trees as a seed treatment, on golf courses, and in commercial or institutional facilities. In short, Acephate is a general use pesticide registered for use on food crops, agriculture seed and non-bearing plants, ant mounds and horticultural nursery[4].Acephate has been registered for use by the U.S. *Environmental Protection Agency* (EPA) since 1973. Acephate has the molecular formula of $C_4H_{10}NO_3PS$.

There are various types of insecticide and pesticides but owing to their easy availability these pesticides are used in the criminal poisoning cases. Pesticide and insecticides are extensively used in agriculture and household remedies for the control of insects and pests. Hence due to their easy availability, inadvertent knowledge and quick action these pesticides are being largely used for suicidal and homicidal purpose.[3] In such cases medical officers preserve proper biological sample for process of

toxicological case work. In such situation toxicologist play a vital role in identification and detection of the toxic substances. In medico legal autopsy case, Organ phosphorus compounds soon became active constituents of several household formulation of for killing bugs and rats. Organ phosphorous poisonings is very common in India (especially in rural area). The organophosphorus poisoning has claimed the largest number of victims in last fifteen years in Maharashtra state and hence in the forensic toxicology. it hasbecome necessary to identify organophosphorus insecticide as a group.

4.1.1 Literature Survey:

In literature, a number of reagents such as mercuric nitrate followed by biphenyl carbazone (Joglekar1968)[5,] mercuric nitrate followed by potassium ferrocyanide (Katkar 1976)[6,] Nessler's or tollens reagent (Kawale 1976),[7] alkaline resorcinol (Geiger,1976), NaOH and ortho toluidine followed by potassium ferricyanide (Lanjewar)[8,] etc has been reported for the detection of organ phosphorus insect ides by thin layer chromatography. E. H. Elgailani *et al.*[9] reported the levels of pesticide residues of acephate 75% SP in some vegetable samples in Albaha local area, Saudi Arabia. But there is no any reference for identification of Acephate.

In existing paper, we develop a new, specific chromogenic spray reagent and new solvent system for the detection and identification of Acephate by HPTLC. The reagent consisting of 0.1% solution of ferric chloride in 80% ethanol with 1% Sulfosalicylic acid in 80% ethanol.Solvent system-(petroleum ether: methanol 95:5)

4.1.2 Present Work:

It is therefore necessary to have a sensitive, specific reagent to detect Acephate in biological materials. We developed a new, specific chromogenic spray reagent and new solvent system for the detection and identification of Acephate by HPTLC. The reagent consisting of 0.1%

solution of ferric chloride in 80% ethanol with 1% Sulfosalicylic acid in 80% ethanol.Solvent system-(petroleum ether: methanol 95:5)

4.1.3 Experimental section:

Chemicals and Reagent

Acetone, methanol, ethanol, petroleum ether, chloroform, sulphosalisilic acid, ferric chloride used were of analytical grade (Merck). Acephate, Endosulfan, Malathion, Phosphomidon, Propoxur, Deltamethrin, Diazepam all standards were available in our laboratory. Accelerated solvent extractor (ASE 200) was used for extraction of Acephate from biological sample distilled water were used throughout.

Spray reagent:

a) 0.1% solution of ferric chloride in 80% ethanol

b) 1% solution of Sulfosalicylic acid in 80% ethanol

Standard solution: Standard solution of Acephate (1 mg/ml) was prepared in methanol. Similarly, separate standard of Endosulfan, Deltamethrin, Propoxur, Malathion, Phosphomidon, Diazepam were also prepared in methanol.

4.1.4 Extraction procedure:

Acephate was extracted from biological sample using accelerated solvent extractor ASE 200 (Dionex). It is an automated system for extracting organic compounds from variety of solid and semisolid samples. If the sample contains water then diatomaceous earth is added to absorb the water content and get a solid orsemisolid sample for extraction. The ASE 200 accelerates the traditional extraction process by using solvent at elevated temperature and pressure is applied in the sample extraction cell to maintain the heated solvent in a liquid state during the extraction. After heating the extract is flushed into the collection vials and is ready for analysis. Approximately 20 gm of visceral sample such as stomach, intestine, liver, and spleen, kidney cut into fine pieces along with liquid and blank viscera were mixed with diatomaceous earth and transferred into the extraction cell. The extracts were collected in a clean collection vial.

Diethyl ether was used for extraction at 50 C at 100 PSI pressure in two cycles. The extract obtained were transformed into steel capsule and evaporated to dryness at room temperature. The residue was dissolved at 2 ml of ethanol and processed further by HPTLC.

4.1.5 High Performance Thin Layer Chromatographic Method:

Chromatography was performed on 20 cm X 20 cm silica gel 60 F HPTLC glass plate (Merck). A camag (Switzerland), Linomat, IV applicator was used to apply 2,4,6 and 10 µl standard solution of Acephate (10 µl) in ethanol equivalent to 10 µg along with extract of viscera, Propoxur(carbamate), Deltamethrin(pyrethroid), Malathion, Phosphomidon(organophosphorus), Endosulfan (Organo chloro insecticide were also applied on HPTLC plate. The plate was then developed in a saturated 24 cm X 8 cm X 22.5 cm Camag twin through TLC chamber to a distance of 10 cm using petroleum ether, methanol (95:5) v/v as mobile phase. The plate was removed from the chamber, dried in air and sprayed with 1% solution of ferric chloride in 80% ethanol and 1% solution of Sulfosalicylic acid in 80% of ethanol. Successively white spot on (pink background) were developed at Rf 0.43 for standard Acephate and viscera having history of death due to Acephate.

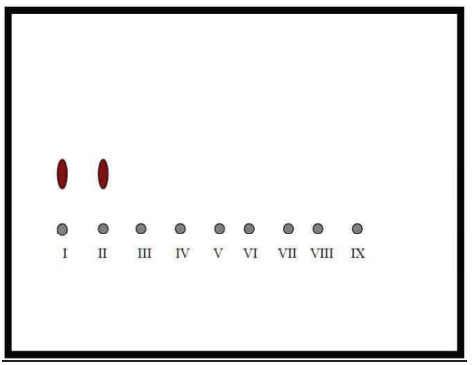

FIGURE 19:HPTLC CHROMATOGRAM

HPTLC Chromatogram obtained from:

I) Standard Acephate II) Acephate from Visceral extract. III) Blank Viscera IV) Malathion V) Phosphomidon (Organophosphorus insecticide) VI) Endosulfan (Organo chloro insecticide) VII) Propoxur (Carbamate insecticide) VIII) Deltamethrin (Pyrethroid insecticide) IX) Diazepam (Drug)

4.1.6 Result and Discussion:

The new developed spray reagent is highly sensitive and specific for the detection of Acephate from biologicalmaterial also the solvent system used in this work is different. No spots were observed for propoxur(carbamate)Deltamethrin(pyrethroid)Malathion(organophosphorus) Endosulfan (Organo chloro insecticide) and Diazepam (drug). Biological impurities such as amino acid, peptides and proteins do not interfere in this spray. This reagent is therefore sensitive and specific for Acephate.

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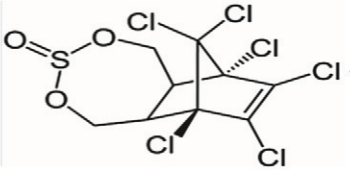
Chapter Iv

High-Performance Thin-Layer Chromatographic Detection of Endosulfan from Biological Samples

Section B



JPC

Short Communications

High-Performance Thin-Layer Chromatographic Detection of Endosulfan from Biological Samples

Ulka K. Kulkarni*, Krishna V. Kulkarni, Rajendra K. Pardeshi, and Dhananjay V. Mane

Key Words:

Endosulfan
Organophosphate
Viscera

1 Introduction

Endosulfan (6,7,8,9,10,10-hexachloro-1,5,5a,6,9,9a-hexahydro- 6,9-methano-2,4,3-benzodioxathiepine-3-oxide; **Figure 1**) is an organochlorine pesticide used for agricultural purposes [1]. It is applied as a fumigant for vegetables, fruits, and cotton. Though it is a highly toxic pesticide, it is easily available in the market of all the parts of India. Due to its easy availability, people frequently misuse this pesticide for suicidal and homicidal purpose [2, 3]. Every year, the Forensic Science Laboratories receives approximately 1000 human and cattle poisoning cases of endosulfan.

High-performance thin-layer chromatography (HPTLC) is the versatile technique for the analysis of a large number of chemical substances, drugs, and pesticides. This technique can be conveniently and easily used for routine toxicological work.

The detection of endosulfan by thin-layer chromatography (TLC) was reported in the literature, by using different chromogenic reagents, *viz.*, alcoholic *o*-toluidine or *o*-dianisidine and irradiation with UV [5], sodium hydroxide followed by methanolic thymol [6], cobalt acetate followed by *o*-toluidine in acidic acid [7], ethanolic diphenyla mine [8], cobalt acetate–sodium hydroxide followed by potassium iodide starch [9], and sodium hydroxide followed by 5% (*w*/*v*) aqueous nickel chloride–30% ammonia (50:50) *v*/*v* [10].

In the present paper, we report the use of 5% NaOH solution and 2% pyridine followed by *p*-aminoazobenzene in acetic acid yielding intense orange color. For better analysis, various solvent systems were used. The best of five solvent systems were selected which are shown in **Table 1.**

Table 1

The selected solvent systems and orange spots observed.

Sr. No.	Pesticide	Solvent systems	R_f value

Section B

4.2 Introduction:

Endosulfan (6,7,8,9,10,10-hexachloro-1,5,5a,6,9,9a-hexahydro-6,9-methano-2,4,3-benzodioxathiepine-3-oxide.

Figure1) is an organochlorine pesticide used for agricultural purposes[1]. It is applied as a fumigant for vegetables, fruits, and cotton. It is sold as a mixture of two different forms of the same chemical (referred to as α- and β-Endosulfan). It is a cream-to-brown-coloured solid that may appear crystalline or in flakes. It has a distinct odour similar to turpentine. The use of Endosulfan is being restricted to certain crops and is scheduled to be cancelled for all uses by 2016. Endosulfan can be released into the air, water, and soil in areas where it is applied as a pesticide. Though it is a highly toxic pesticide, it is easily available in the market of all the parts of India. Due to its easy availability, people frequently misuse this pesticide for suicidal and homicidal purpose. [2,3] Every year, the Forensic Science Laboratories receives approximately 1000 human and cattle poisoning cases of Endosulfan.

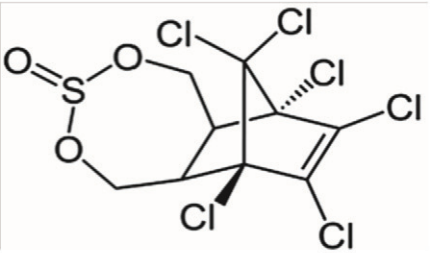


FIGURE 1: CHEMICAL STRUCTURE OF ENDOSULFAN

Forensic toxicologists need to be able to characterize this insecticide. There are many modern analytical methods for the identification and detection of Endosulfan in biological samples, such as ultraviolet (UV), high-performance liquid chromatography (HPLC), gas chromatography (GC),

gas chromatography–mass spectrometry (GC–MS), etc.; although these methods are very rapid, specific and sensitive; they cannot be always used for the detection of insecticides which are extracted sensitive and accurate, they are highly expensive and also time-consuming.[4]

High-performance thin-layer chromatography (HPTLC) is the versatile technique for the analysis of a large number of chemical substances, drugs, and pesticides. This technique can be conveniently and easily used for routine toxicological work. In the present study, HPTLC technique is used for detection and identification of Endosulfan from biological materials.

4.2.1 Literature Survey:

The detection of Endosulfan by thin-layer chromatography (TLC) was reported in the literature, by using different chromogenic reagents, *viz.,* alcoholic *o*-toluidine or *o*-dianisidine and irradiation with UV [5] , sodium hydroxide followed by methanolic thymol[6] , cobalt acetate followed by *o*-toluidine in acidic acid [7], ethanolic diphenyl amine[8] , cobalt acetate–sodium hydroxide followed by potassium iodide starch [9], and sodium hydroxide followed by 5% (*w*/*v*) aqueous nickel chloride–30% ammonia (50:50) *v*/*v*.[10]

Malve *et al*.[11] have reported the thin layer chromatographic technique for detection and identification of Endosulfan insecticide in biological materials. A new chromogenic spray reagent *m*-dinitrobenzene was used. Reaction of Endosulfan with alkali releases sulphur trioxide which reacts with *m*-dinitrobenzene in dimethyl sulfoxide for form a purple violet complex.[27]

4.2.2 Present work:

The increasing number of biological samples for poison detection, there is a need of versatile, sensitive and selective reagent. In a search for a selective and sensitive reagent, alkaline hydrolysis of pyridine with p-amino azobenzene was found to be suitable for detection and identification of Endosulfan in routine forensic toxicological analysis. High Performance

Thin Layer Chromatography (HPTLC) is the method of choice because of its speed, low cost and versatility.

In the present paper, we report the use of 5% NaOH solution and 2% pyridine followed by *p*-amino azobenzene in acetic acid yielding intense orange colour. For better analysis, various solvent systems were used. The best of five solvent systems were selected which are shown in **Table 6.**

4.2.3 Experimental section:

All the chemicals and reagents were of an analytical grade. Distilled water was used throughout.

a) 10% Aq. sodium hydroxide: 10% (*w*/*v*) sodium hydroxide was prepared by dissolving 10 g of sodium hydroxide pellets in 100 mL of distilled water

b) Pesticide standards solution: 10 mg of each pesticide (Endosulfan, DDT, and endrin) was dissolved in 10 mL of ethanol (1 mg mL^{-1}).

c) *p*-Amino azobenzene reagent: 0.25% (*w*/*v*) PAAB in 25% of ethanol.

4.2.4 Extraction Procedure:

An automated system is known for extracting organic compounds from a variety of solid and semisolid samples. If the sample contains water, then diatomaceous earth is added to absorb the water contents and get a solid and semisolid sample for extraction. Accelerated solvent extractor ASE 200 (Dionex) was used for the extraction of viscera. The ASE 200 accelerates the traditional extraction process by using solvent at elevated temperature. Pressure is applied to the sample extraction cell to maintain the heated solvent in a liquid state during the extraction. After heating, the extract is flushed into the collection vials and is ready for analysis. An amount of approximately 20 g of visceral sample, such as stomach, intestine, liver, spleen, and kidney having a history of consumption of Endosulfan pesticide, was cut into fine pieces along with diatomaceous earth and transferred into the extraction cell. The extract was collected in a clean collection vial, and diethyl ether was used for extraction at 50°C and 1000 psi pressure in two cycles. The extract obtained was transferred into a

steel capsule and evaporated to dryness at room temperature. The residue was dissolved in 2 mL of ethanol and processed further by HPTLC.[2,10]

4.2.5 High-Performance Thin-Layer Chromatography:

Chromatography was performed on 10 cm × 10 cm silica gel 60 F_{254} HPTLC glass plate (Merck, Darmstadt, Germany). A Linomat IV applicator (CAMAG, Muttenz, Switzerland) was used to apply spot of standard Endosulfan, DDT, endrin, extract of viscera having a history of death due to the consumption of Endosulfan, blank viscera, Profenophos (organophosphate), and cypermethrin (pyrethroid) on HPTLC plate **(Figure 2).** The plate was then developed in presaturated (24 cm × 8 cm × 22.5 cm) CAMAG twin-through TLC chamber to a distance of 10 cm, using hexane–acetone (80:20) *v*/*v*, hexane–acetone (90:10) *v*/*v*, acetone–benzene (80:20) *v*/*v*, cyclohexane–chloroform (60:40) *v*/*v*, and benzene methanol (40:60) *v*/*v* as the mobile phase. The plate was removed from the chamber, dried in air and sprayed with 5% sodium hydroxide and then 2% pyridine. The plate was allowed to stand for 5 min and then it was sprayed with *p*-amino azobenzene reagent using a glass sprayer. Successively, orange spots were observed at R_f values shown in Table 6.

The selected solvent systems and orange spots observed at below Rf Values

Table 6

SrNo.	pesticide	Solvent system	Rf. Value
1	Endosulfan	Hexane–acetone (80:20)	0.97
2	Endosulfan	Hexane–acetone (90:10)	0.78
3	Endosulfan	Acetone–benzene (80:20)	0.91
4	Endosulfan	Cyclohexane–chloroform (60:40)	0.97
5	Endosulfan	Benzene–methanol (50:50)	0.95

Endosulfan insecticide

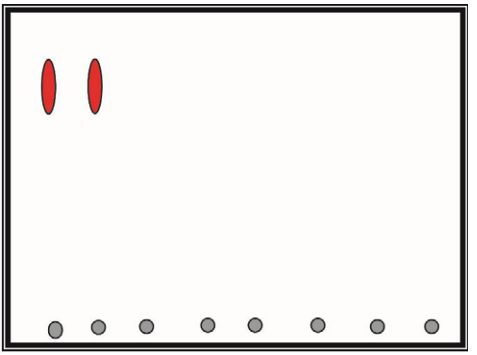


FIGURE 20: HPTLC CHROMATOGRAM OF ENDOSULFAN

HPTLC chromatogram obtained from: I) standard Endosulfan, II) Endosulfan from visceral extract, III) blank viscera, IV) DDT, V) endrin, VI) Profenophos (organophosphorus insecticide), and VII) cypermethrin (parathyroid insecticide, VIII) diazepam (drug).

4.2.6 Recovery Experiments:

Endosulfan (1 mg) was separately added to the minced visceral tissue (50 g), mixed well, and left to stand for 24 h. The tissue samples were then processed as above (extraction procedure) except that the residues from the extraction of the tissue were dissolved in 1 mL of ethanol. This 10 mL solution was spotted on a separate activated plate with the respective 10 µL standard solution of Endosulfan containing 7, 8, 8.5 9, 9.5, and 10 mg in 10 mL ethanol. The plates were then developed and processed as described above [11]. The intensity of the spot obtained for the extracts of the visceral tissue was compared with that from the standard and found to be most similar to the spot resulting from the 9 mg (10 mL) $^{-1}$ standard solution of Endosulfan. Hence, the recovery for Endosulfan was 90%.

4.2.7 UV Spectrophotometry:

On an activated TLC plate, 10 spots each of 20 µL of Endosulfan in ethanol along with a spot of standard Endosulfan sample were placed. The plate was developed as described under HPTLC procedure. A portion of the standard Endosulfan spot was sprayed by this reagent where another portion of the plate was protected by covering it with an uncoated glass plate. The parallel portion to the orange spot (Endosulfan) of the protected

silica layer was scraped off and eluted with 20 mL of ethanol. The solvent was evaporated at room temperature and the residue was then dissolved to the minimum known volume (0.5 mL) in ethanol. The UV absorption spectra for eluent in ethanol were recovered on Specord spectrophotometer with reagent blank which showed absorption at 490 nm. It showed that the linear minimum concentration of Endosulfan gives a positive result with this reagent.

4.2.8 Results and Discussion:

Endosulfan containing cyclic sulphite in its structure is readily hydrolysed by alkali sulphite (characteristic formation from tetravalent sulphur compounds by the action of alkali). The hydrolysis of Endosulfan results in forming chloroform and the chloroform in turn reacts with pyridine to produce Schiff base glutanane aldehyde. This Schiff base of glutanane aldehyde further reacts with *p*-amino azo benzene reagent to produce dark orange coloured spot (the chemical reaction is presented in **Figure 3**). This reagent can be routinely used for the identification of Endosulfan in biological materials in forensic toxicological work.

In the present paper, an effort has been made to use various solvent systems for effective analysis.The reported reagent is selective for Endosulfan, within the group of pesticides. Other organophosphates, organochlorines, carbamate, and parathyroid pesticides did not give coloured spot. For homicidal, suicidal, and accidental purpose, the use of pesticides in India is very common due to their easy availability.

An organochlorine group has large application in the field of agriculture and it is highly toxic for animals and human beings. Nowadays, Endosulfan is largely used for the protection of crops. The increasing number of biological samples for poison detection needs a versatile, sensitive, and selective reagent. The reagent described here is sensitive and selective for Endosulfan and, hence, can be routinely used for the detection and semi quantitative determination of residual Endosulfan in biological and nonbiological materials in forensic toxicology.

Endosulfan insecticide

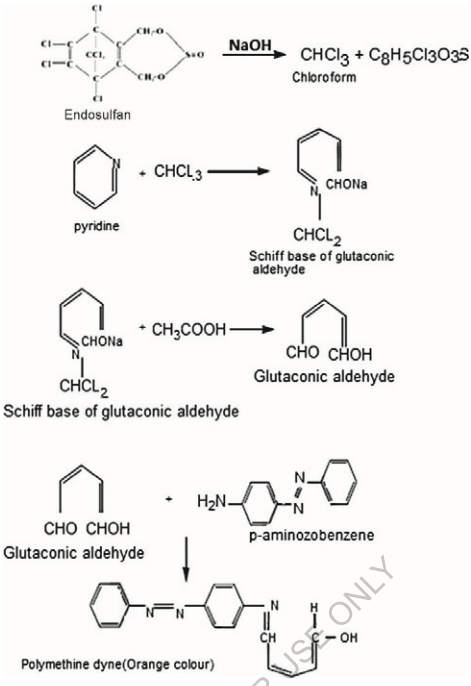


FIGURE 21: PROBABLE CHEMICAL REACTION

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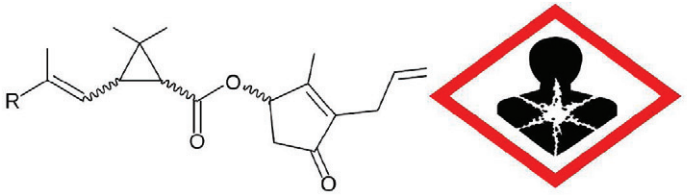
Chapter IV

HPTLC Detection of Pyrethroids in Autopsy Tissues

Section B



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HPTLC Detection of Pyrethroids in Autopsy Tissues

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Abstract Pyrethroids use as an insecticide has been increasing in recent years as a replacement for organophosphate insecticides that are being phased out because of water-quality concerns (California Department of Pesticide Regulation, 2005). Pyrethroids are used in both agricultural and urban (commercial and residential) areas. The

4.3 Introduction:

Pyrethroids are synthetic or semisynthetic compounds related to the natural insecticides pyrethrins. Traditionally, pyrethroids have been classified in two groups depending on their structure: type I and type II, where the difference is an additional cyano group in type II (see Fig.1). The use of both types as pesticides is extensive around the world. For instance, they are commonly used in agronomics (on both crops and stored grain) and with veterinarian purposes.

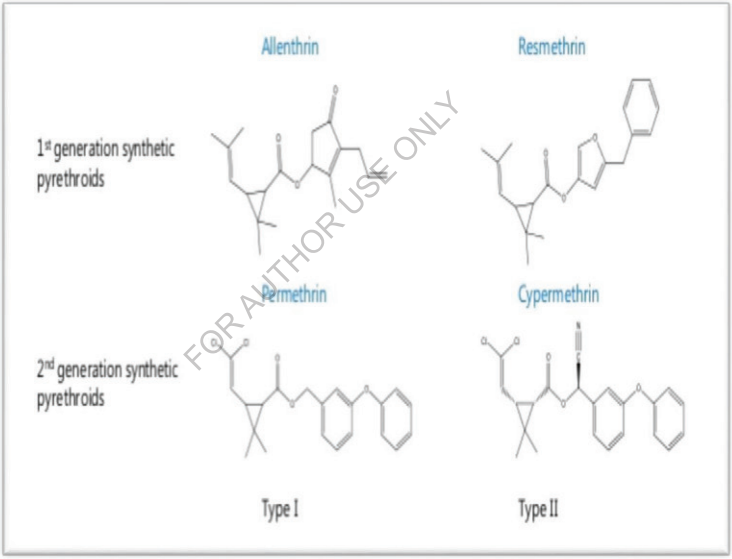


FIGURE 1: CHEMICAL STRUCTURE OF PYRETHROIDS

Pyrethroid use as an insecticide has been increasing in recent years as a replacement for organophosphate insecticides that are being phased out because of water-quality concerns (California Department of Pesticide Regulation, 2005). Pyrethroids are used in both agricultural and urban (commercial and residential) areas.[1] Theoccurrence of pyrethroids is of concern because pyrethroids are known to be highly toxic to aquatic

organisms. Due to their easy availability, Pyrethroids insecticides are often misused in homicidal and suicidal cases, requiring toxicological examination. Forensic toxicologists need to be able to characterize these insecticides.

Cypermethrin, Deltamethrin, fen valerate are the synthetic pyrethroids having insecticidal activity against a wide range of pests with low mammalian toxicity. Highly potent Pyrethroids like cypermethrin, Deltamethrin, Fenvalerate, are the esters of 2,2 dimethyl cyclopropane carboxylic acid with 2,2-dihalovinyl side chain, these Pyrethroids have a cyanide group attached at the α-position to the carboxyl ate group.

As pyrethroid use continues to increase in both urban and agricultural settings, it is important to have robust, sensitive, rapid methods that are capable of detecting and measuring these compounds in autopsy tissues with relevant concentrations (below acute toxicity levels) in both blood and viscera. HPTLC detection of Pyrethroids from autopsy tissues is best method for forensic case work, where more than thousand autopsy samples are tested every month. This method will also help scientists to understand pyrethroid behaviour in the environment. Very few TLC methods have been utilized for the presence of pyrethroid insecticide from autopsy tissues.

4.3.1 Literature survey:

The literature survey reveals that, the reagents include Phosphomolybdic acid [2], ultraviolet (UV) irradiation [3]and silver nitrate, and irradiation with UV light [4], palladium Chloride[5] etc. None of above mention chromogenic reagent is specific for α-cyno ester compound. However, Copper-acetate and O-tolidine[6]and P-nitro benzaldehyde followed by P-dinitrobenzene [7] reagents have been recently reported. In the present with the slight modification for very much similar compound is reported.

4.3.2 Present work:

We report, alkaline hydrolysis of p-nitro benzaldehyde as a specific spray reagent for α cyno ester by High Performance Thin Layer

Chromatography. The basic of this reagent underlines on the formation of well-known chemical reaction of Benzoin condensation. This reagent produces violet spots relatively with syntheticPyrethroids containing cyno group.

4.3.3 Experimental section:

Chemicals and Reagents:

All the chemicals and reagents used were of analytical grade.

1) Sodium hydroxide solution: 10%-10 gm of sodium hydroxide pallets in distilled water and dilute to 100 ml.
2) P-Nitro benzaldehyde reagent:1%-1 gm of P-Nitro benzaldehyde dissolved in 100 ml of ethanol
3) Reference Standard of insecticides: 1mg/ml $^{-1}$ prepared of –Pyrethroids in Ethanol

A) Cypermethrin in Ethanol
B) Deltamethrin in Ethanol
C) Fenvalerate in Ethanol

- Solvent: Hexane, acetone, Toluene, Cyclohexane

4.3.4 High Performance thin Layer Chromatography:

Chromatography was performed on 20 cm x 20 cm silica gel $60F_{254}$ HPTLC glass plate (Merck), A camag (Switzerland), Linomat IV applicator was used to apply 10 □l in ethanol equivalent to 10 µg along with std cypermethrin, Deltamethrin, Fenvalrate and extract of negative control tissue of autopsy were also applied on HPTLC plate. The plate was then developed in pre-saturated 24 cm x 8 cm x 22.5 cm camag twin through TLC chamber to a distance of 10 cm using hexane: acetone (8:2) and Cyclohexane: Toluene (5:5) v/v as mobile phase. The plate was removed from the chamber, dried in air and sprayed with sodium hydroxide solution and P- Nitro benzaldehyde reagent by using glass sprayer. Successively blue-violet spots were observed at RF values shown in table 7.

Table 7

Control Insecticides	Solvent system Hexane: acetone {8:2)	Solvent system Cyclohexane: Toluene

		(5:5)
1. Cypermethrin	0.53	0.60
2. Deltamethrin	0.60	0.93
3. Fenvalrate	0.66	0.64

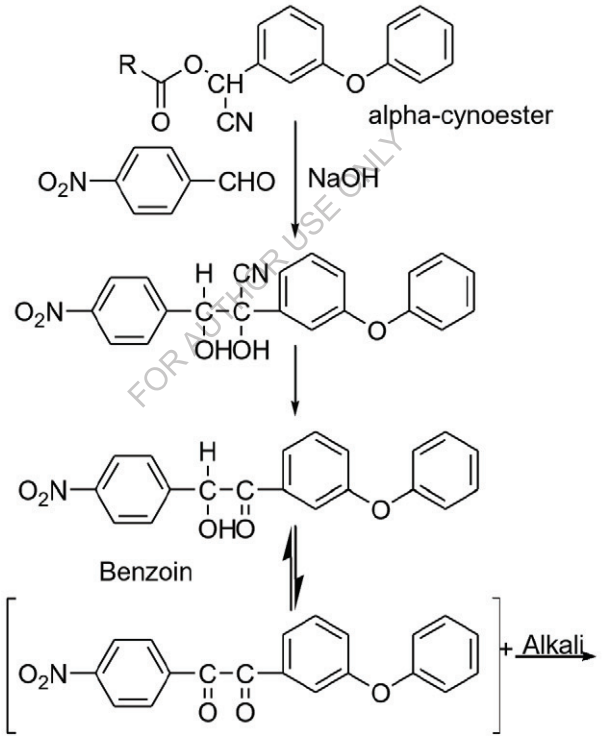
4.4.4 Extraction of Pyrethroids in Autopsy tissues:

100 gm of stomach, Intestine, Liver, Spleen or Kidney tissue (having a death history of Cypermethrin poisoning) was chopped to fine pieces in a beaker. About 20 gm of ammonium sulphate was added and it was kept for two hours for precipitation of proteins and amino acids. About 100 ml of diethyl ether or hexane was added to the beaker, stirred well and watery part was transferred to a 500 ml of glass separating funnel. After waiting for few minutes, the organic solvent layer was separated in glass capsule and evaporated in air. The procedure was repeated for at least two times with enough quantity (2*100 ml) of the solvent for complete extraction of the Pyrethroid, the Concentrated residue

4.4.5 Result and Discussion:

The result of HPTLC screening is tabulated in Table 1. Which shows RF values of Cypermethrin, Deltamethrin, Fenvalrate insecticides. The developing solvent mixtures were: 1) Hexane: Acetone 2) Cyclohexane: Toluene (5:5)

The reaction mechanism of pyrethroids shows—alkaline hydrolysis of α-cyno ester produce HCN and corresponding benzaldehyde derivative [8]. Sodium Hydroxide present in the reaction mixture readily reacts with pyrethroid compound to produce HCN. Which reacts with p-Nitro benzaldehyde reagent: to give Benzoin Condensation product. The Benzoin condensation is reversible in alcoholic alkaline solution through tautomerism. A characteristic colour reaction is observed on addition of aqueous alkali to a solution of Benzoin in the presence of air (autoxidation) [9]. After a was dried over sodium sulphate and then it was applied to the HPTLC plate for screening along with a reference standard.



the certain induction period a solution acquires blue-violet colour, which disappears when the solution is oxygenated by keeping for some time.

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Chapter VI

Summary and Conclusions

Introduction:

The use of forensic science offers new possibilities to meet various challenges in the field of law and judiciary. Forensic science is the application of a broad spectrum of sciences to answer questions of interest to a legal system. This may be in relation to a crime or a civil action. Forensic toxicology has developed as a forensic science in recent years and is now widely used to assist in death investigations, in civil and criminal matters involving drug use, pesticides and other toxic compounds, plants toxins etc. The incidence of poisoning in India is among the highest in the world, and it is estimated that more than 50,000 people die every year from toxic exposure. The causes of poisoning are many - civilian and industrial, accidental and deliberate. The commonest agents of poisoning in India appear to be pesticides (organophosphates, carbamates, chlorinated hydrocarbons, and pyrethroids), sedative drugs, drugs most commonly targeted include amphetamines, benzodiazepines, cannabis, cocaine and the opiates, but can be any other illicit substance or almost any over-the-counter or prescribed drug, as well as poisons available to the community. chemicals (corrosive acids and copper sulphate), alcohols, plant toxins (datura, oleander, strychnos, and gastro-intestinal irritants such as castor, croton, Calotropis, etc.), and household poisons (mostly cleaning agents). Therefore, with the ever-increasing cases of poison in India, the role of forensic toxicology has been greatly appreciated in various cases. The discipline requires high level skills in analytical techniques with a solid knowledge of forensic toxicology and chemistry.

In the recent years in India, the use of different types of insecticides, fungicides, rodenticides and herbicides is increased in agriculture to protect the crops and commercial plants from insects to get good yield and also, they are often used in houses to kill the mosquitoes, cockroaches, bed bugs and rats. Easy availability of these insecticides frequently misused in suicidal or homicidal poisoning. Forensic toxicology is an important branch of forensic Science. The number of fatal poisoning

cases received for toxicological analysis is constantly increasing. The poison isolated from biological material in poisoning cases generally is in microgram quantities.

Hence, in the present thesis an attempt has been made to develop new technique for identification and detection of drugs and forensic interest compounds by using Thin Layer Chromatography (TLC) and High-Performance Thin Layer Chromatography (HPTLC). These methods are highly sensitive and can be used for unequivocal identification of pesticides/drugs from biological and non-biological materials. These methods are discussed in five chapters.

Competent Components of Thesis:

In each chapter, a new chromogenic spray reagent is developed for identification of pesticides from biological materials.

Carbamates (carbaryl, propoxur, carbofuran) belong to a family of chemicals that kill or control the insect known as carbamate. These insecticides are widely used against a broad spectrum of insects on field crops, fruits, and vegetables and against household flies and mosquitoes. New varieties of these insecticides are easily available. Due to their easy availability, insecticides are often misused in homicidal and suicidal cases, requiring toxicological examination.

Forensic toxicologists need to be able to characterize these insecticides. Hence, in routine forensic toxicological examination, high-performance thin-layer chromatography (HPTLC) is the best technique for the identification and detection of insecticides from biological materials.

We developed A Specific Spray reagent for the identification and detection of carbaryl in biological materials. In present work, we reported, the use of 10% NaOH solution followed by a mixture of sodium bromide and copper chloride for high-performance thin-layer chromatographic (HPTLC) detection and identification of carbaryl insecticide with a solvent system hexane and ethyl acetate (9:1).

The study combined, for the first time, forensic investigation, chemistry and botany to create a unique platform needed for the

identification of poisonous plants and their components in forensic exhibits, blood, urine, stomach wash and viscera. The research was focused on the poisonous plants previously detected at the laboratory, as well as the requests received for the analysis of multi/toxic plant components.

In present thesis, a method developed for detection of Cannabis by HPTLC which found to be high -throughput, sensitive, reproducible and cost-effective compared to other methods. Although the instrumental methods are sensitive, they are expensive but there are limitations to their use in routine forensic work owing to the large number of samples (involving urine samples) to be handled. A number of chromogenic reagents have been reported. In a search for an alternative chromogenic reagent, P-Anisidine reagent in combination with ammonium metavanadate was found to be suitable for the detection of cannabinoids in marijuana.

Development of method for isolation and identification of newly invented pesticides have been described in in this study. Acephate is an insecticide that belongs to the organophosphate group of chemicals. We developed a new, specific chromogenic spray reagent and new solvent system for the detection and identification of Acephate by HPTLC. The reagent consisting of 0.1% solution of ferric chloride in 80% ethanol with 1%Sulfosalicylicacidin80%ethanol. Solvent system-(petroleum ether: methanol 95:5).

The increasing number of biological samples for poison detection, there is a need of versatile, sensitive and selective reagent. In a search for a selective and sensitive reagent, alkaline hydrolysis of pyridine with p-amino azobenzene was found to be suitable for detection and identification of Endosulfan in routine forensic toxicological analysis. High Performance Thin Layer Chromatography (HPTLC) is the method of choice because of its speed, low cost and versatility.

Pyrethroid use as an insecticide, has been increasing in recent years as a replacement for organophosphate insecticidesas pyrethroid use continues to increase in both urban and agricultural settings, it is important

to have robust, sensitive, rapid methods that are capable of detecting and measuring these compounds in autopsy tissues with relevant concentrations (below acute toxicity levels) in both blood and viscera. HPTLC detection of Pyrethroids from autopsy tissues is best method for forensic case work, where more than thousand autopsy samples are tested every month. This method will also help scientists to understand pyrethroid behaviour in the environment. Very few TLC methods have been utilized for the presence of pyrethroid insecticide from autopsy tissues. We report, alkaline hydrolysis of p-nitro benzaldehyde as a specific spray reagent for α-cyno ester by High Performance Thin Layer Chromatography. The basic of this reagent underlines on the formation of well-known chemical reaction of Benzoin condensation. This reagent produces violet spots relatively with synthetic Pyrethroids containing cyno group.

Hence in the present, an author, being a part of forensic laboratory found that there are so many problems for identification of various poisoning substances in biological and non-biological materials. There are no previous references for identification of such types of cases. By doing research and development work, and using the spray reagent developed by us, these cases are solved. Thus, the innovative methods applied by the author facilitated the identification of modern-day insecticides and pesticides. This is the boost to forensic toxicology division to enhance the analytical methodologies by which the challenge of finding out modern day poisons can be achieved.

Major Conclusions:

- The objective of the present study is to develop new methods for identification of drugs and forensic interest compounds from biological and non-biological materials.
- Most commonly targeted drugs include amphetamines, benzodiazepines, cannabis, cocaine and the opiates. Forensic interest compounds are different types of insecticides, fungicides, rodenticides and herbicides.

- 10% aq. sodium hydroxidewitho-Toluidine reagent followed by 10% aq. sodium nitrate: New chromogenic reagent for detection of carbamate insecticides.
- For carbaryl insecticides: use of 10% NaOH solution followed by a mixture of sodium bromide and copper chloride is a specific chromogenic reagent to identify carbaryl in autopsy tissue.
- This study combined, for the first time, forensic investigation, chemistry and botany to create a unique platform needed for the identification of poisonous plants and their components in forensic exhibits, blood, urine, stomach wash and viscera. The research was focused on the poisonous plants previously detected at the laboratory, as well as the requests received for the analysis of multi/toxic plant components.
- HPTLC detection of cannabis reported by developing new chromogenic reagent as sodium hydroxide with saturated aqueous ammonium metavanadate solution followed by p-Anisidine reagent.
- The reagent consisting of 0.1% solution of ferric chloride in 80% ethanol with 1% Sulfosalicylic acid in 80% ethanol found sensitive, specific reagent to detect Acephate in biological materials.
- Alkaline hydrolysis of p-nitro benzaldehyde as a specific spray reagent for α cyno ester by HPTLC. This reagent produces violet spots relatively with synthetic Pyrethroids containing cyno group.

Future Perspectives:

From the present work, it is revealed that using the spray reagents developed by us, forensic cases can be solved. Thus, the innovative methods applied by the author facilitated the identification of modern-day insecticides and pesticides. This is the boost to forensic toxicology division to enhance the analytical methodologies by which the challenge of finding out modern day poisons can be achieved. But still, various insecticides are also available in market. The increasing number of biological samples for poison detection, there is a need of versatile, sensitive and selective reagent. Work on different insecticides and poisonous plants and its reagents is in progress. The author hopes to report soon when work is over.

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